Current Status of Anti-Tuberculosis Therapy: A Patent Analysis

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Abstract: Affecting more than one third of the world population, tuberculosis remains one of the world’s most dreadful diseases, with no easy cures. Mycobacterial infestation and the evasion of host immune response are significantly responsible for the emergence of pulmonary tuberculosis. Mycobacterium tuberculosis, a weak gram positive, facultative aerobe colonizes in respiratory regions rich in oxygen reserves. Up regulation of CR and MR expression and function due to signaling by LAM results in eliciting immuno regulatory cytokines IL-4, IL-8 and Th2. Binding of NF-xkB complex with mRNA prevention, due to mutation of leucine zipper domain of IK, inhibits the activation of cytokines and receptor molecules. Mechanism of energy generation by conversion of ADP to ATP, initiated by utilizing intermediary and/or end products of carbohydrate, amino acid or fatty acid catabolism is essential in approximating potential drug targets for elimination of the bacterium. A few improved diagnostic techniques have been evaluated over the last few years like Interferon Gamma Release Assays, Nucleic Acid Amplification tests etc. of which most have certainly proven to facilitate specific detection of TB. Drugs like Rifampicin, Isoniazid etc. have also shown great curing effects on TB patients.

Further research is required for better understanding of mechanism of pathogenesis and multiple drug resistance issues for developing the effective therapeutics and diagnostics against TB. The paper focuses on the effective diagnostics and therapeutics applications for tuberculosis prevention and cure based on recent patents and their analysis.

Keywords: Biochemical pathways, ethambutol, glyoxylate shunt, isoniazid, lipid metabolism, Mycobacterium tuberculosis, NF-B signaling, pyrazinamide, rifampicin, TCA cycle.

INTRODUCTION

Over the last few decades, across both developing and industrialized countries, an increased emergence of drug resistant strains of Mycobacterium tuberculosis has been observed, along with a widespread synergy of Mtb with the human immunodeficiency virus (HIV) [1]. Tuberculosis (TB), because of its unique features i.e. potential for developing resistance to strongest antibiotics, poses a major threat to the antimicrobial techniques that have been or are being developed by researchers or scientists to combat the lethal disease. Cell dormancy, very slow growth rate, complexity of cell envelope, intracellular pathogenesis, homogeneity in the genetic makeup are among the few characteristic features that happen to be common for most of the Tubercle bacilli [2]. The pace of infection gives tuberculosis bacteria time to mutate and thus increasing the possibility of evolution-based antimicrobial resistance. As a result, multi-drug resistance develops, making it an issue of serious concern. Hence, the need of hour is to discover new drugs or methods that could combat the lethal condition of multi-drug resistance in various strains Mycobacterium tuberculosis [3]. The disease caused by inhaling the airborne causative agent marks its onset by fever, cough, and difficulty in breathing and inflammatory infiltrations. Formation of tubercles takes place along with caseation, pleural diffusion and fibrosis. Tuberculosis can also be caused as result of infection by M. bovis, M. microti, and M. africanum, apart from M. tuberculosis, which is known to be the main causative agent. Mycobacterium tuberculosis (MTB) is a non-motile, non sporulating, aerobic and weak gram positive. It is acid fast bacteria which appear to be straight or slightly curved rod shaped under the microscope [4]. The cell wall of MTB is one of the major determining factors of its virulence. The structure of the cell wall consists of three major components, namely, mycolic acids, cord factors and Wax-D. The mycolic acid molecules are of primary interest because of their deadly qualities whereas the waxy cell wall confers many unique characteristics like extreme hydrophobicity, resistance to drying, acidity or alkalinity along with many antibiotics and distinctive immuno stimulatory properties. Mycolic acids are unique to Mycobacterium and Corynebacterium [4].

The main proteins and protective antigens of Mycobacterium tuberculosis are a triad of related gene products called
the antigen 85 (Ag85) complexes. Each has also been implicated in disease pathogenesis through its fibronectin-binding capacities [5]. Gram staining is used to categorize the bacteria based on the make-up of their cell walls. The amount of the polymer that peptidoglycan forms is a mesh-like structure and determines whether or not the bacterium can hold onto the stain administered in gram staining. MTB cannot be classified as truly gram positive or negative as its cell wall is not affected by gram staining because of the high content of lipids, mostly mycolic acid and lipids. Hence MTB, which is an acid-fast bacterium and whose acid-rich cell walls retain a red dye, is used for staining, in spite of attempts at De-colorization [6]. The Genome of Mycobacterium tuberculosis has a G + C-rich genome, which was first, published in 1998 by Sanger Institute (H37Rv strain) [7]. The cell wall of MTB contains an additional layer beyond the peptidoglycan, which is exceptionally rich in unusual lipids, glycolipids and polysaccharides [8]. Novel biosynthetic pathways generate cell-wall components such as mycolic acids, mycocerosic acid, phenolthiocerol, lipoarabinomannan and arabinogalactan [9]. Several of these may contribute to mycobacterial longevity, trigger inflammatory host reactions and act in pathogenesis.

The pathology of cell wall of MTB is responsible for pathogenic properties. Infectious particles when reach the alveoli sacs in the lungs, macrophages engulf the bacteria and clump together into granulomas in order to contain the infection. The infestation pathways are later discussed in the paper in detail. In spite of the possibility of extrapulmonary TB, most of the cases occur in the upper lungs, which follow the activation of dormant MTB. When MTB becomes active, its cell wall plays a major role in replication and resistance to immune responses. After the initial infection, MTB can reproduce within the macrophage until the cell bursts, which alerts macrophages from peripheral blood. However, this process continues since the newly produced MTB cannot be fully destroyed by the macrophages [10]. As MTB replicates further, T-cell lymphocytes are activated by major histocompatibility complex molecules, which allow the T-cells to recognize MTB antigens. At this point cytokines are released, which activate the macrophages, which are cell-mediated immune response, which opposes the original antibody mediated immune response, and allow them to destroy MTB. At this stage, small rounded nodules known as tubercles form and create an environment where MTB is unable to multiply. However, it is because of its cell wall that MTB can survive in the low pH and anoxic tubercles for long periods of time [11]. These tubercles are surrounded by many inactivated macrophages in which MTB is able to replicate. Although the cell-mediated immune response is capable of destroying individual bacterium, it is also responsible for the growth of tubercules, which occurs when MTB replicates within and subsequently ruptures inactivated macrophages. In these ways, the cell wall of MTB allows it to escape or complicate each step of the immune process, creating a need for man-made antibiotics.

**PATHOGENIC INFESTATION, REGULATION AND BIOGENESIS OF LIPID METABOLISM**

*Mycobacterium tuberculosis* (MtB) infects an individual intracellularly forming caseous lesions. The pathogen adheres to lung tissues rich in oxygen reserve by entering the body through the respiratory tract. From the site of infestation, the bacterium spreads to lymph nodes and other parts of the body via blood and lymphatic system [12] Fig. (1). Demonstrates the pathogenic progression of the bacterium and the air borne transfer of bacterium from a patient to a healthy individual. In *M. tuberculosis* infection, macrophages are the key effectors for eliciting immune response.

Interaction of macrophage with *Mycobacterium* has a critical role in eliciting immune response. The events, which lead to the development of active tuberculosis (TB) infection, could be summarized as:

1. **Mechanism of bacterial binding:** The primary immune reaction elicited by the body in response to an MtB infection is the phagocytosis of bacterium by activated alveolar macrophages, termed as professional antigen presenting cells (APCs) by binding of *M. tuberculosis* to macrophages via cell surface receptor molecules. Mycobacterial surface glycoprotein lipoarabinomannan (LAM) mediate the interaction between mannose receptor (MR) present on surface phagocytic cells and mycobacteria. Interleukin (IL-4), Th2-type cytokine and prostaglandin E2 (PGE2) up regulates MR expression and function [13]. Bacterial antigens bind to the antigenic receptors that force the bacterial fragments into a phagosome. The essential role of CD4+ T-cells, in protection against *M. tuberculosis* infection, as illustrated by the diagram, is the production of interferons (INF-γ) and tumor necrosis factor alpha (TNF-α) [13].

2. **Prevention of lysosomal action:** Phagocytosed microorganisms are then supposed to undergo phagolysosomal fusion and get degraded by lysosomal acidic hydrolases. This highly controlled event establishes a substantial inhibitory mechanism to evade lysosomal fusion. Sakamoto et al. [14] theorized that the survival of *M. tuberculosis* is ensured inside the macrophage by the prevention of phagolysomallysosomal fusion.

3. **Signaling pathway inhibition:** Survival of MtB inside macrophage by mutation in leucine zipper domain of Ik kinase (IkK). Nuclear factor kappa light chain enhancer of activated B-cell (NF-kB) pathway inhibition proves to be an efficient strategy for the prevention of mRNA down regulation. NF-kB pathway is stimulated by signals such as cytokines, free radicals, viral and bacterial antigens [14]. NF-kB proteins controls transcription of DNA, which results in, modified cellular response. As depicted in Fig. (2). NF-kB pro-
tein is present in the cytosol linked with inhibitory protein IB. Extracellular stimuli such as tumor necrosis factor (TNF-α) are the prime activator of IkB kinase (IkK). Activation of NF-kB protein occurs by the tagging of IkBα fragment by IkK. The ubiquitin tag designates the IkBα fragment to be sent and degraded in the proteasome. Activated NF-kB is translocated into the nucleus. The p50 and p65 domains of NF-kB bind to response elements, which are specific DNA sequences. This leads to the transcription of DNA to mRNA to protein. The protein demonstrates cellular changes, which display immune responses towards the external stimulus.

Infection of cell by Mycobacterium tuberculosis results in mutations in leucine zipper domain of IkK thereby inhibit-
ing the degradation of IkB by proteasome [14]. Since the inhibitory protein remains bound to the NF-B complex, the translocation and binding of p50 and p65 onto the DNA sequence is prevented. Consequently the downstream of DNA to protein is averted. The cell becomes incompetent in exhibiting immune response towards the bacterial infection. Development of target drug aimed at the degradation of IkB thereby aiding the binding of transcription factor to the mRNA can facilitate the elicitation of immediate immune responses.

Other mechanism of phagosomal-lysosomal fusion evasion: Anti-mycobacterial effector functions of macrophages including generation of reactive oxygen intermediates (ROI), reactive nitrogen intermediates (RNI) and cytokine mediated responses, have facilitated M. tuberculosis in evading degradation by lysosomal hydrolase [13].

4). Responses of immune system: Cell-mediated immunity (CMI) is known to develop within 2 to 6 weeks of infection. At the site of infestation, dead macrophages and lymphocytes enter into the caseous lesions. Thereafter, the bacterium stops growing exponentially and the dead APCs cluster to expand the lesion. Fate of the bacterium depends upon the immune response elicited by the host body. Caseous necrosis of bronchi and cavitations resulted in sacking of individual’s respiratory system thereby causing death. The bacterium can release into the environment after enormous increase in number consequently infecting other healthy individuals. Due to excessive mechanisms of resistance, the immune response elicited by the macrophages proves to be a week barrier against the spreading of bacilli into peripheral systems. During the terminal stages of infection, fibrosis occurs at the circumference of the granuloma so as to prevent the bifurcation of infection into the internal organs [13].

MECHANISM AND POTENTIAL DRUG TARGETS IN THE CITRIC ACID CYCLE (TCA) CYCLE AND GLYCOSYLATED SHUNT

Imitating metabolic processes of host functions is a crucial feature of the pathogenicity of Mycobacterium tuberculosis. M. tuberculosis depends on a variety of carbon sources for growth, but evidence by Marrero et al. [15] has linked fatty acids as the primary basis of energy during Mtb infection. Fat catabolism involves the breakdown of glycerides and triglycerides into simpler fatty acids. This process is accomplished by β-oxidation of triglycerides liberating acetyl-CoA, which eventually enters the citric acid cycle.

The bacterium also enters the TCA cycle as a result of protein catabolism and carbohydrate catabolism. Bacterial metabolisms involving 1) fatty acid catabolism 2) tricarboxylic acid cycle (TCA) and 3) gluconeogenesis, have been discussed, wherein the knowledge of pathways undertaken by the bacterium is used in understanding the pathogenic progression of disease. The technical knowhow of disease evolution also aids the development of new drug therapies. As discussed by Marrero et al. [15] fatty acids synthesis during in vivo growth, provides precursor for the tricarboxylic acid cycle (i.e. pyruvate) and gluconeogenesis, which is essential for growth of the bacterium.

Tricarboxylic acid cycle is a cascade of reaction engaged by M. tuberculosis to produce energy via dehydrogenation of pyruvate, thereby releasing energy in the form of adenosine tri phosphate (ATP). Citric acid, which derives the name of the cycle is consumed and regenerated in the process of one complete cycle. Key features of the citric acid cycle are consumption of acetate in the form of acetyl-CoA, reduction of nicotine-adenine dinucleotide (NAD+) and removal of carbon dioxide. The oxidation of TCA cycle intermediates; to prevent loss of carbon in the form of CO₂ is prevented by glyoxylate shunt, which bypasses the TCA cycle [15]. Figure 3 demonstrates the series of reactions involved in the TCA cycle and glyoxylate shunt.

Murthy et al. [16] in due course of study on TCA cycle undertaken by Mtb have commented on the activity of each enzyme involved in the series of reactions, wherein they have explained the effect of substrate and enzymes on the production of intermediates and final products as well as on the uptake rate of oxygen. As stated by Murthy et al. pyruvate, oxaloacetic acid, acetate and lactate significantly stimulated the oxygen uptake above the endogenous value. Succinate, fumarate, c-oxoglutarate, malate and glyoxylate increased the oxygen uptake to only a very small extent.

FUNCTIONS OF INDIVIDUAL ENZYMES INVOLVED IN THE TCA CYCLE

The precursor of TCA cycle as demonstrated in Fig. (3), was pyruvate, which converted to citrate by the activity of pyruvate-dehydrogenase, citrate synthase, NADP⁺ and CoA [17]. Isocitrate also catalysed the reduction of NAD⁺ to NADPH forming oxalosuccinate using the enzyme isocitrate dehydrogenase, which is a branching point for glyoxylate shunt. The formation of 2-oxoglutarate, reduced NAD⁺ to NADH demonstrating the presence of NAD⁺ dependent oxoglutarate dehydrogenase to form succinyl-CoA. The 2-oxoglutarate-dehydrogenase system plays an important role in facultative anaerobic respiration thus sensitivity of 2-oxoglutarate-dehydrogenase system to oxygen deficiency and glucose repression can lead to the enzyme complex being used as a potential drug target. The formation of intermediary products like succinyl-CoA, succinate, fumarate and malate via succinyl-CoA synthetase and succinate dehydrogenase culminates in the formation of oxaloacetate, which re-enters the chain to form citrate [17].

Regulation of TCA cycle: The regulation of the TCA cycle depends on substrate availability and product inhibition. Murthy et al. [16] in their study stated that the NADH-oxidase, NADPH oxidase and transhydrogenase had a great controlling effect on the respiration by regulating the amounts of the available oxidized coenzymes, the presence of these enzymes in M. tuberculosis H37R, were examined. The cell-free extracts of M. tuberculosis oxidized only a few intermediates of the tricarboxylic acid cycle. However, the
fact that citrate, aconitate and isocitrate, which were not utilized and which often showed slight inhibition of the endogenous respiration of the intact cells, were oxidized by cell-free extracts in the presence of an artificial electron acceptor offers definite evidence that the cells of this bacillus were impermeable to these substrates. Though the cell-free extracts of *M. tuberculosis* have all the normal components of the tricarboxylic acid cycle, the dehydrogenases of the cycle, unlike most other biological systems, are all NADP+ dependent, with the exception of malate dehydrogenase, which shows activity with NAD+ at a high pH.

From the above observations, it can be concluded that TCA cycle proceeded in a cyclic manner under aerobic conditions, but under anaerobic conditions it gets modified to enter a non-cyclic, branched pathway.

Youmans *et al.* [18] studied the effects of individual compounds of TCA cycle on the growth of *M. tuberculosis*. Growth was obtained in the presence of certain concentrations of glycerol, lactic acid, pyruvic acid, acetic acid, oxalosuccinic acid, a-ketoglutaric acid, and oxalacetic acid [19]. With the exception of acetic acid and oxalosuccinic acid, these compounds stimulated the growth of the organisms to the same degree, as did the higher concentrations of glycerol. However, the generation time obtained with acetic and oxalosuccinic acids were similar to the generation time found with the lower concentrations of glycerol. The theory that a tricarboxylic acid or a similar cycle may be involved in the carbohydrate metabolism of *M. tuberculosis var. hominia* since pyruvic, acetic, oxalacetic, oxalosuccinic, and a-ketoglutaric acids supported the growth of the virulent *H37Rv* strain [19]. As stated by Youmans *et al.* a number of enzymes of the Kreb's cycle which appear to be present in mycobacteria were, aconitase, isocitric dehydrogenase, oxalosuccinic carboxylase, ketoglutaric dehydrogenase, malic dehydrogenase, fumarase and succinic dehydrogenase [19].

**Fig. (3). TCA cycle and glyoxylate bypass mechanism** - Mechanisms of *M. tuberculosis* initiated by utilization of pyruvate from carbohydrate catabolism and/or acetyl-CoA from amino acid or fatty acid catabolism and approximating potential drug targets for elimination of *M. tuberculosis*. (Adapted from Murthy *et al.* 1962) (Ref: [16]). The NADH generated by the TCA cycle initiates electron transport pathway, oxidative phosphorylation thereby generating ATP. In *M. tuberculosis*, due to the absence of mitochondrion, the TCA reaction cascade occurs in the cytosol.
POTENTIAL DRUG TARGETS FOR ELIMINATION OF M. TUBERCULOSIS FROM LUNGS

Genetic modifications, which prevent the inhibition of NF-B, thereby aiding the binding of transcription factor to the mRNA, can prove to be essential drug targets against the Mtb infection. Studies by Singh et al. also explained the development of anti-tuberculous drugs for persistent bacilli. As stated, the glyoxylate bypass was essential for persistent bacilli; interference with it holds the potential for designing new antibacterial drugs. Singh et al. developed kinetic models of the TCA cycle and glyoxylate bypass in Escherichia coli (E. coli) and M. tuberculosis, and examined the effects of inhibition of various enzymes in the M. tuberculosis model [19]. Metabolic control analysis (MCA) was done to give an insight into the cellular effect of inhibition of a particular enzyme. Elimination of M. tuberculosis could be achieved by two main mechanisms, which were, decreasing the flux through an essential metabolic pathway to a nonviable level, or increasing the concentration of a metabolite to a toxic level. The M. tuberculosis model study concluded that the deletion of both isocitrate lyase (icl) genes led to the elimination of the bacilli from the lungs [19].

Singh et al. [19] simulated the M. tuberculosis model and concluded that inactivation of both isocitrate dehydrogenase (icd) 1 and isocitrate dehydrogenase 2 is essential for the proceeding of glyoxylate bypass in persistent mycobacteria, wherein increasing the amount of active isocitrate dehydrogenases can stop the flux through the glyoxylate by pass [19]. Hence potential drug target against persistent mycobacteria could be the kinase that inactivated isocitrate dehydrogenase 1 and 2. Hence, the competitive inhibition of isocitrate lyases along with a reduction in the inactivation of isocitrate dehydrogenases, both combined could be an effective drug target areas. Kinetic modeling of biochemical pathways was an important tool to assess various potential anti-tuberculosis drug targets that interfere with the glyoxylate bypass flux, and indicated the type of inhibition needed to eliminate the pathogen. The advantage of such an approach to assess drug targets was that it explained the systemic effect(s) of the modulation of the target enzyme(s) at cellular level. MCA elucidated the mechanism involved in elimination of M. tuberculosis by specific enzyme inhibition [19].

IMPROVED DIAGNOSTIC TECHNIQUES FOR TUBERCULOSIS

It is very difficult to accurately detect cases of positive TB, many a times there is occurrence of false positives and negatives. The currently known tests for diagnosis of TB are based exclusively on immunological tests assessing host-specific cellular immunity to MTBC antigens. Even though the detection of TB cannot be very accurate, there are various techniques that are currently used to detect the same, and these techniques do prove to be helpful in most of the cases.

1. Interferon Gamma Release assay (IGRA)

This has been the biggest advancement in the field of detection of tuberculosis, the development of T-cell based IGRA. This technique is dependent on early secretion of Early Secretion Antigen Target (ESAT-6) and Culture Filterate Protein (CFP-10) by M. tuberculosis antigens in the host system, so that interferon-gamma production starts to take place. Blood tests are done to detect these antigens and hence the detection positive MTB in the sample [20].

2. Automated Cultures

Since liquid cultures are more sensitive than WHO has recently released a policy statement to replace solid cultures by liquid ones in all those tests that are based on solid cultures [e.g. Lo¨wenstein-Jensen (LJ)] [20].

3. Nucleic Acid Amplification Tests (NAATs)

NAATs have been evaluated for detection of TB for nearly 2 decades now, and several meta-analysis shows that these have more specificity than other detection methods. These use either PCR or transcription mediated amplification to detect mycobacterial nucleic acids [20].

MUTATION OR INACTIVATION OF GENES: POTENTIAL DRUG TARGETS IN M. TUBERCULOSIS MODEL

Drugs and Therapies Involved in the Effective Treatment of M. tuberculosis

Clinical drug-resistant TB mainly occurs as a result of man-made selection during treatment of these genetic alterations through erratic drug supply, suboptimal physician prescription and poor patient adherence [18].

Though TB has been known to develop resistance to drugs over a period of time, there has been some success in developing some antibacterial agents for effective treatment of TB. The following drugs are currently employed in the fight against TB, and have proven to be effective in most of the cases, if administered in the right way. The program of ‘DOTS’, being run by WHO also includes some of the drugs mentioned below.

Mechanism and method of drug therapies- Currently, a four-drug treatment is used consisting of INH, RIF, pyrazinamide and ethambutol. Resistance to first line anti-TB drugs has been linked to mutations in at least 10 genes; katG, inhA, ahpC, kasA and ndh for INH resistance; rpoB for RIF resistance, embB for EMB resistance, pncA for PZA resistance and rpsLand rrs for STR resistance [21].

Isoniazid

INH enters the cell as a prodrug that gets activated by an enzyme catalase peroxidase encoded by katG gene. The peroxidase activity of this enzyme is necessary to activate INH to a toxic substance in the bacterial cell [22]. This toxic substance subsequently affects the mycolic acid biosynthesis...
which is an important component of the cell wall. A lack of mycolic acid synthesis will result in the loss of cellular integrity and thus the bacteria dies. Middlebrook et al. [23] demonstrated that a loss of catalase activity can induce in INH resistance. Kas gene encodes a $\beta$-ketoacyl-ACP synthase involved in the biosynthesis of mycolic acids. Mutations have been found in this gene which shows low levels of INH resistance.

Rifampicin (RIF)

RIF interferes with transcription by the DNA-dependent RNA polymerase. It binds with the $\beta$-subunit of the RNA polymerase, disrupting the transcription and thereby killing the organism [24].

Pyrazinamide (PZA):

PZA is a prodrug which gets converted to its active form, pyrazinoic acid (POA) by the pyrazinamidase (PZase) encoded by pncA gene. The activity of PZA is highly specific for M. tuberculosis. It is only active against M. tuberculosis at acidic pH when POA gets accumulated in the cytoplasm due to an ineffective efflux pump which results in the lowering of intracellular pH such that it inactivates a vital fatty acid synthase [24]. Therefore, Mutations in the pncA gene correlates with resistance to PZA.

Ethionamide (ETH)

ETH is a prodrug that is activated by bacterial metabolism. The activated drug causes disruption in cell wall biosynthesis by inhibiting mycolic acid synthesis. Mutations in the promoter of the inhA gene are associated with resistance to INH and ETH [25].

D-Cycloserine (DCS)

DCS is a cyclic analog of D-alanine which helps in cross linking of peptidoglycan assembly. DCS competes with D-Alanine for the enzymes D-alanyl-D-alanine synthetase (Ddl) and D-alanine racemase and therefore inhibiting the cell wall synthesis [25].

PATENTS ON DIAGNOSIS OF TUBERCULOSIS

Some impactful and newly granted patents on diagnosis of tuberculosis have been discussed below:

While other non-impactful granted patents on diagnosis of cancer have been cited in Table 1. Further, some newly granted patents on diagnosis of tuberculosis have been discussed and summarized below.

The invention by Kamerbeek et al. is based on the DNA polymorphism present on chromosomal locus direct repeat (DR) region, which is exclusively present in M. tuberculosis genome. Majorly M. tuberculosis contains one or more IS6110 elements in the DR region of genome. Kamerbeek et al. sequenced whole of the DR region of M. tuberculosis H37Rv along with flanking regions adjacent to the DR region. For identifying an infected individual, presence of spacer nucleic acid, prior to amplification was determined. If this sequence was found to be homologous with the amplified product, the presence of M. tuberculosis was confirmed in the individual [26].

The initiative by Mcallister et al. is the invention of ‘identification kits’ for detection of M. tuberculosis infection. The kit works on the principle of identification of oligonucleotide sequences present on bacterial genome and amplifying M. tuberculosis 16S rRNA. The amplification of specific nucleic acid or ‘target sequence’ is done to increase the sensitivity of diagnostic without compromising specificity for the same. Nucleic acid hybridization is carried out for detection and quantitation of specific nucleic acid sequences. The invention is largely convenient which is why it is used for diagnostic testing [27].

The method involving the administration of therapeutically effective amount of a polypeptide into a subject has been devised by Lewinsohn et al. The methods is based on

<table>
<thead>
<tr>
<th>Regulation mechanisms</th>
<th>Effect of chemical messengers</th>
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<tr>
<td>1. ADP</td>
<td>Reduction in a threshold level of ADP resulted in the accumulation of precursor NADH and also inhibited a number of enzymes</td>
</tr>
<tr>
<td>2. NADH</td>
<td>Excess NADH inhibited pyruvate dehydrogenase, isocitrate dehydrogenase, a-ketoglutarate dehydrogenase and also citrate synthase</td>
</tr>
<tr>
<td>3. Acetyl-CoA</td>
<td>Inhibited he pyruvate dehydrogenase</td>
</tr>
<tr>
<td>4. Succinyl-CoA</td>
<td>Inhibited a-ketoglutarate dehydrogenase and citrate synthase.</td>
</tr>
<tr>
<td>5. ATP</td>
<td>Inhibited citrate synthase and a-ketoglutarate</td>
</tr>
<tr>
<td>6. Calcium</td>
<td>Activated pyruvate dehydrogenase phosphatase, isocitrate dehydrogenase and a-ketoglutarate dehydrogenase, consequently pyruvate dehydrogenase complex</td>
</tr>
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Table 1. Several points of regulation and effect of chemical messengers on regulation points.

(Adapted from Doelle HW, 2003) (Ref. [17]).
the ‘in vitro assays’ for detecting the presence of CD8+ T cells in a sample followed by in vivo assays to detect a delayed type hypersensitivity reaction. Individuals with LTBI were defined as healthy people despite the positive TST with no indications of active infection. The specimen from an infected individual was studied by screening of T cell clones against a genomic peptide library, screening of ex vivo GD8+ T-cells against a genetic peptide library, additional screening of T cell clones against a genomic peptide library [28].

A number of fragments (peptide epitopes) from three *M. tuberculosis* cell wall proteins were classified as early antigens that induce antibodies during early stages of *M. tuberculosis* infection in humans. Laal et al. identified the proteins as Proline-Arginine Repetitive Protein (PTRP), PE--PGRS51, and LipC. The invention was based on the early diagnosis of *M. tuberculosis* infection with the help of a single peptide, peptide mixture in the form of fusion polypeptides or peptide multimers. The proteins were useful as antigens to check for the presence of antibodies against these proteins. This was a rapid and easy technique, which reduced the dependence on conventional modes of *M. tuberculosis* infection identification. The above peptides are now used as immunogens for TB vaccines [29].

Affecting nearly 8.8 million new people, and resulting in 3 million annual deaths across the world, the disease of tuberculosis remains to be a major threat to human mortality. The classical way to diagnose tuberculosis is the sputum smear test, although early tuberculosis goes undetected in most of the cases. A precise and a less time consuming method for detection of tuberculosis is vital; hence Roland et al. in this paper have written about their findings on the identification of nucleotide sequences that would distinguish between an infection arising from *M. tuberculosis* and infections arising from other Mycobacterium species (e.g. *M. canetti*) [30].

Covering a diverse cell types, introduction of recombinant DNA technology has led to molecular cloning of several coding sequences. The production of products encoded by such sequences is done by the transfer of expression vectors, which are inserted with genes of interest, into the host cell. Here the contribution Jeffery et al. talk about recombinant nucleic acid molecules that encode fusion polypeptides comprising a Ral2 polypeptide and a heterologous polypeptide, fusion polypeptides, expression vectors and host cells comprising the nucleic acid molecules [31].

Skeiky et al. have devised fusion protein coding sequence to encode a protein product to be used as immunogen to stimulate or enhance immune reaction to *M. tuberculosis* infection. Coding sequence were modified by polymerase chain reaction (PCR) and cloned into an expression plasmid. Each plasmid contained three coding sequences ligated end to end. In particular the above invention relates to fusion polypeptides that contains *M. tuberculosis* antigen. The fusion proteins induced both T cell and B cell reactions. Further the fusion protein is used with adjuvants to prompt both cell-mediated and humoral immunity towards *M. tuberculosis* infection [32].

Elsa et al. have compared three methods for the detection of *M. tuberculosis* antigens i.e. tuberculin skin test or PPD which was compared to MAPIA assay for detecting antibodies in host plasma and MADA used for detecting proteins expressed in vivo in host plasma or serum by pathogen in the healthy individuals whereas MAPIA and MADA of diseased individuals were compared to clinical diagnosis. The method involves 1) Contact with a sample of a biological fluid like serum sputum, pleural fluid, urine etc. with a solid support. 2) Add an amount of first antibody against at least one *M. tuberculosis* protein. 3) Do the screening for the presence of *M. tuberculosis* proteins in the biological fluid by adding an amount of a second antibody, which will bind to first antibody [33].

Lindner et al. gave a method to detect the presence of one or more mycobacteria of *M. tuberculosis* complex by checking the presence of nucleic acid under conditions that do not detect the presence of nucleic acid. Detecting and amplifying nucleic acid wherein the *M. tuberculosis* complex organism is selected from *M. tuberculosis*, *M. bovis*, *M. africanum*, *M. canetti*, *M. microti* or a combination thereof, followed by performing polymerase chain reaction (PCR) to thereby detect nucleic acid comprising a sequence of at least 20 contiguous nucleotides in length [34].

The inventors sought to identify a biomarker of TB infection by one or more of the organisms of the *M. tuberculosis* complex that is expressed i.e. at the protein level and/or RNA level.

Patients infected with *M. tuberculosis* or suffering from the symptoms of *M. tuberculosis* infection could be detected and treated using current methods of diagnosis and therapeutics. Symptoms involved in diagnosis, which are not limited to chronic cough, were blood streaked sputum, fever, chest pain, pallor, chills, fatigue, night sweats, and weight loss. Kandror et al. stated in their study that if *M. tuberculosis* infection spread to organs other than the lungs, a variety of organ specific symptoms could arise that were particular to the organ infected. Test and diagnostic tools that aided in a diagnosis of infection included x-rays, chest x-rays, tuberculin skin test, blood tests, microscopic examination of bodily fluids, microbiological culture of bodily fluids, chest photofluorography, the Ziehl-Neelsen stain, auramine-rhodamine stain, fluorescent microscopy and PCR [35].

Lalvani et al. have developed a rapid detection method of *M. tuberculosis* infection by highly accurate blood test. This is the first method to distinguish between BCG-vaccinated and *M. tuberculosis* infected patients and is based on the exposure of the host to mycobacterium that expresses ESAT 6 sensitive detection of antigen specific T cells from host with one or more selected peptides or analogues that can further bind T cell receptor and recognizes any selected peptides or analogues [36].
Depending upon various culture conditions such as growth substrate, temperature, oxygen availability etc., different microorganisms utilise different substrates to satisfy their energy and nutritional needs, and release various primary and secondary metabolites during their metabolism. Some of these metabolites formed readily volatilize at low temperatures, known as MVOCs (microbial volatile organic compounds). The main idea of Stephen et al. was to use biomarkers for identifying bacterial pathogens, particularly, the detection and identification of certain volatile compounds as markers for Mycobacterium tuberculosis or M. bovis, by analysis of a gaseous sample [37].

Hacick et al. have provided a set of at least butylatedhydroxytoluene or 4, 6-di (1,1 -dimethyl) -2-methyl -phenol for breath analysis for the diagnosis and monitoring lung cancer.

This is done by collecting a breath sample from a lung cancer and determining and comparing the levels of volatile organic compounds in the breath sample from the lung cancer patient to the levels of volatile organic compounds in a control sample; and identifying compounds having levels that are significantly different in the breath sample from the lung cancer patient as compared with the control sample, thereby identifying a set of volatile organic compounds indicative of lung cancer consists at least one of butylatedhydroxytoluene and 4,6-di((1,1-dimethyl)ethyl)-2-methyl-phenol [38].

Mudgel et al. have devised a method for identifying M. tuberculosis in a sample detecting one or more volatile organic compounds indicative of a presence of or response to treatment or resistance of the M. tuberculosis in the sample, the one or more volatile organic compounds being selected from the group consisting of ethyl propionate, 1-pentanol, methyl valerate, 1-hexanol, ethyl `valerate, methyl caproate, ethyl caproate, and any of the foregoing compounds in isotopically labeled form also combination of two or more volatile organic compounds is indicative of the presence of or response to treatment or resistance of M. tuberculosis in the sample [39].

One third of the world’s population suffers from latent TB; active TB prevails in many geographic regions. Current methods involved in the diagnosis aren’t very precise and time consuming. Most deaths by tuberculosis can be controlled by early detection and treatment. The work conducted by Fernandez-Reyes et al. provides a method of diagnosing tuberculosis (TB) in a test subject, said method comprising: (i) providing expression data of two or more markers in a subject, wherein at least two of said markers are selected (e.g. C-reactive protein (CRP), serum amyloid A (SAA), serum albumin, apolipoprotein-A1 (Apo-A1)); and (ii) comparing said expression data to expression data of said marker from a group of control subjects, wherein said control subjects comprise patients suffering from inflammatory conditions other than TB, thereby determining whether or not said test subject has TB [40].

Kathrick invention uses mass spectrometry-based methods for the detection of mycolic acids to be used as biomarkers to determine the tuberculosis infection. It involves testing a sample for the presence of Mycobacterium tuberculosis by preparing a mycolic acid profile using using electrospray ionization mass spectrometry wherein the presence of C26-a-mycolic acids is indicative of the presence of Mycobacterium tuberculosis. A test sample taken from an infected individual after treatment can be assessed, and the profile of mycolic acids can be compared to the profile of mycolic acids in a positive sample. A linear increase in MA levels was observed with increasing numbers of bacterial cells, for both sputum and medium [41].

The initial interaction between the microorganism and the host dramatically influences the outcome of the mycobacterial infection, either proceeding towards the pathogen containment or establishment of the tubercular pathology. The bacteria slowly grow in vitro and the current methods used for diagnosis of TB have the disadvantage of giving false positives and being expensive. This paper highlights the work of Giulia et al. relating to the use of gene sequences specific Mycobacterium tuberculosis and their corresponding proteins for the diagnosis and the prevention of the tubercular infection [42].

Keertan et al. described a method which used antibodies to detect the presence of specific biomarkers. Binding of the antibodies to the biomarkers indicated active extra pulmonary tuberculosis in an infected patient. The indicator may be a chromatographic, fluorescent, optical, enzyme-linked or radio-labeled indicator. It can also distinguish between active and latent tuberculosis which is important for determining treatment of the patient. Most importantly, this method can provide us with the results within the matter of minutes, unlike other tests, which need to incubate the sample for more than 12 hours [43].

Namba et al. have provided a method for the detection of MPT64 antigen in a biological sample for the diagnosis of infection with M. tuberculosis complex with higher accuracy. Further they provided that MPT64 can be detected in biological sample with high sensitivity by an immunoassay without culturing or after culturing, before Mycobacterium tuberculosis starts growing so that diagnosis can be done more rapidly and safely. In addition to it, immune chromatographic test strip can be used for diagnosis of infection with M. tuberculosis and thus ensures that risk of secondary infection is reduced [44].

Gennaro et al. have discovered that the genome of Mycobacterium tuberculosis contains an open reading frame (ORFs) which have been deleted from the avirulent BCG strain, thus these ORFs are ‘M. tuberculosis BCG Negative polymapeutics’ (MTBN). They provided that the MTBN polypeptide elicited a skin response in animals infected with M. tuberculosis or M. bovis but not in animals sensitized to either BCG or M. avium. Therefore proteins incorporated by ORF’s present in the genome of M. tuberculosis but absent from genome of BCG represent reagents help in discriminat-
ing between them especially for diagnostic methods like skin tests and in vitro assays for M. tuberculosis specific antibodies. This discriminates between subject exposure to M. tuberculosis and vaccination with BCG. Thus invention features many polypeptides, functional segments such as DNA molecules having the polypeptides or the functional segments, vectors containing DNA molecules, cells transformed by the vectors, functional segments, or DNA molecules and variety of diagnostic, therapeutic methods, which utilizes the foregoing [45].

PATENTS ON THERAPEUTICS OF TUBERCULOSIS

Some impactful and newly granted patents on diagnosis of tuberculosis have been discussed below: While other non-impactful granted patents on diagnosis of tuberculosis have been cited in Table 2A, 2B. Further, some newly granted patents on therapeutics of tuberculosis have been discussed and summarized below.

The main feature of the present invention by Montenes et al. reported an isolated microorganism belonging to the M. tuberculosis complex that comprised of the inactivation of the Rv0757 gene that conferred a PhoP- phenotype and the inactivation of the fad D26 gene that prevented the DIM production (DIM- phenotype). Furthermore, the present discovery provided a detailed description of the use of this microorganism for the formulation of a vaccine for the prevention and cure of tuberculosis [46].

Scholler’s invention described the use of MHC-peptide complexes comprising M. tuberculosis antigenic peptides and its use in the diagnosis of, treatment of or vaccination against a disease in an individual. Interactions between peptide encoded by the Major Histocompatibility Complex and T-cell receptors (TCR) were required to elicit specific immune responses. It primarily required activation of T-cells so that the peptides were presented to the T-cells by the MHC complexes? An MHC multimer comprised of subunits (a-b-P)₁₅, wherein n > 1, wherein a and b together formed a functional MHC protein capable of binding the peptide P, wherein (a-b-P) is the MHC-peptide complex formed when the peptide P binds to the functional MHC protein [47].

Pulmonary tuberculosis, caused by M. tuberculosis in humans, largely infects and localized to the lung. BCG, the attenuated form of Mb, is the only licensed human vaccine against TB, and provides only partial protection against TB. This paper is the work of Charles et al. focusing on immunizing an individual against M. tuberculosis, by a method that administers to the subject an immunologically effective amount of a first antigen from the M. tuberculosis or a first polynucleotide encoding the first antigen and administering to the lung of the subject an immunologically effective amount of a second antigen from the Mycobacterium or a second polynucleotide encoding the second antigen [48].

The term nucleic acid immunization used by Michael et al. refers to the introduction of a nucleic acid molecule encoding one or more specific antigens into a host cell for the in vivo expression of the antigen or antigens. The nucleic acid fragment derived from M. tuberculosis could be directly introduced into the recipient by typical intramuscular or intradermal injection, transdermal particle delivery, and inhalation or by oral, intranasal or mucosal methods of administration. The molecule alternatively can be injected ex vivo into cells, which have been removed from a patient. In the ex vivo case, cells containing the nucleic acid molecule of interest were reintroduced into the subject such that an immune response could be elicited against the antigen encrypted by the nucleic acid molecule [49].

Beltz et al. isolated and purified non-peptide antigens from M. tuberculosis. The invention described the vaccine compositions used to elicit an immune response specific for M. tuberculosis. Also, it related to immunogenic or vaccine compositions comprising of at least one non-peptide antigen isolated, wherein the isolated non-peptide antigen evoked a specific immune response against M. tuberculosis, which could further comprise one or more T-cell stimulating compounds [50].

Weimer et al. presented a therapeutic composition having at least one syringopeptin and at least one rhamnolipid so that any of the following activities were maintained: antibacterial; antifungal; and antitumor activities. A therapeutic composition comprising: an effective amount of a syringopeptin; an effective amount of a rhamnolipid; and a pharmaceutically acceptable carrier. The present innovation illustrated a method for inhibiting or treating microbial infection in a subject by either providing a subject in need of inhibition or treatment of cancer or a microbial infection, monitoring a specific amount of therapeutic composition to the subject so as to inhibit or treat the microbial infection [51].

Typical embodiments of the invention by Harth et al. were the utilization of antisense polynucleotides to modify the expression and function of M. tuberculosis genes comprising of glutamine synthetase, araA, ask, groES and the genes of the antigen 85 family or complex. The inventors demonstrated the feasibility of utilizing antisense PS-ODNs as antimicrobial agents against this pathogen. The inventors stated that the modified PS-ODNs inhibited the expression of M. tuberculosis glutamine synthetase significantly and the reduction in enzyme activity correlated to a reduction in the amount of the polyLglutamate/glutamine structure in the mycobacterial cell wall and with substantial inhibition of bacterial replication [52].

Nikolaevna et al. have described the use of substituted ethylene diamines having improved anti-mycobacterial activity treating infectious diseases with the help of amino alcohol pre-loaded resins and amino acids as linker. Ethambutol (EMB) is a widely used antibiotic for the treatment of TB, with over 300 million doses delivered for tuberculosis therapy in 1988 [53].

Alland et al. use the molecular beacon probes to detect Rifampicin-resistant M. tuberculosis which accomplished the necessity for a rapid single PCR assay in identification of Rifampicin-resistant M. tuberculosis with RRDR (rifampicin resistance-determining region) mutations that were high on
Table 2A. Recent patents issued or published on diagnosis of tuberculosis [26-45].

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Assignee/Applicant</th>
<th>Inventor</th>
<th>Patent Number</th>
<th>Title</th>
<th>Issued/Published</th>
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<tr>
<td>[30]</td>
<td>Institute Pasteur, Veterinary Laboratories Agency</td>
<td>Roland <em>et al.</em></td>
<td>WO2003070981A2</td>
<td>Sequences specifically deleted from <em>Mycobacterium tuberculosis</em> genome and their use in diagnostics and as vaccines</td>
<td>Nov, 2004</td>
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<tr>
<td>[42]</td>
<td>Cappelli Giulia, Colizzi Vittorio, Consiglio Nazionale Ricerche, Mariani Francesca</td>
<td>Giulia <em>et al.</em></td>
<td>WO2005021790A2</td>
<td>Use of gene sequences specific for <em>Mycobacterium tuberculosis</em> and their related proteins for diagnosis and prevention of tubercular infection</td>
<td>Mar, 2005</td>
</tr>
<tr>
<td>[43]</td>
<td>University of Cape Town</td>
<td>Keertan <em>et al.</em></td>
<td>WO2010070581 A1</td>
<td>Methods and device for diagnostics tuberculosis</td>
<td>Jun, 2010</td>
</tr>
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</table>
Table 2B. Recent patents issued or published on therapeutics of tuberculosis [46-65].

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Assignee/Applicant</th>
<th>Inventor</th>
<th>Patent Number</th>
<th>Title</th>
<th>Issued/Published</th>
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<tr>
<td>[52]</td>
<td>University of California, Gunter Harth, Marcus A Horwitz, David Tabatabadze, Paul C Zamecnik</td>
<td>Harth et al.</td>
<td>WO2002094848A1</td>
<td>Treatment of Mycobacterium tuberculosis with antisense oligonucleotides</td>
<td>Nov, 2002</td>
</tr>
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</table>
both sensitivity and specificity. The events involve amplifying a nucleic acid containing the RRDR of the rpoB gene in a patient sample followed by hybridizing the amplified nucleic acid and then conducting melting temperature (Tm) analysis to determine a Tm value for each probe. Finally; comparing the Tm value for each of the molecular beacon probes that was greater than the Tm value for the wild type RRDR would indicate the presence of rifampicin resistant \textit{M. tuberculosis} [54].

Gennaro \textit{et al.} have devised a method in which \textit{M. tuberculosis} polypeptide and genes encoding them were used in diagnostic and prophylactic methodologies. The invention was based on the discovery of a novel group of open reading frames (ORFs) encoding polypeptides that are secreted by \textit{M. tuberculosis}. An isolated DNA molecule consisting of a DNA sequence encoding a polypeptide with an amino acid sequence selected from the group consisting of the amino acid sequences of the polypeptides. The portion of the DNA molecule encoded a segment of the polypeptide shorter than the full-length polypeptide, and the segment had \textit{M. tuberculosis} specific antigenic and immunogenic properties [55].

In non-replicating persistence stages, survival of \textit{M. tuberculosis} can be determined due to its tolerance to drugs; being the possible reason for the long-term treatments. Inclusion of rifampin and pyrazinamide in the multidrug cocktail because those drugs significantly accelerated the clearance of the infection was a major breakthrough in the treatment of TB. Now, the development of new combination therapy regimens, including drugs that are bactericidal against these NRP stages of \textit{M. tuberculosis} has the potential for shortening the length of treatment. Madrid \textit{et al.} in their paper, mentioned about Metronidazole, a 5-nitroimidazole, one of the first compounds that was found to be bactericidal towards NRP \textit{M. tuberculosis}. The activity of this compound against \textit{M. tuberculosis} had been determined using a minimum inhibitory concentration (MIC) measurement. The toxicity and selectivity of the compounds had been quantified by a 50% cytotoxicity concentration (CC50) using a Vero cell viability assay with the Promega Cell Titer Glo reagent [56].

Mainardi \textit{et al.} have stated that infection with \textit{M. tuberculosis} usually resulted in primary tuberculosis or Ghon's complex in approximately 90% of the individuals. In more than half of the infected individuals, activation of dormant bacteria happens within the first 2 years following the time of infection, but this activation may not occur for a very long time, in some cases for up to 5 years following the time of infection. Several \textit{M. tuberculosis} organisms remain viable for life, a condition known as latent or dormant tuberculosis. In this paper, the author explained a method for the screening of antibacterial substances comprising a series of steps, one of them being the determination of the ability of a candidate substance to inhibit the activity of a purified L, D-transpeptidase enzyme from \textit{M. tuberculosis} having at least 90% amino acid sequence identity with the amino acid sequence of SEQ ID No 1 [57].

With an increasing threat of tuberculosis infections across the world, the development of multi drug resistant mycobacteria is of extreme danger. This paper by Makarov \textit{et al.}, threw light on benzothiazin derivatives and their use as antibacterial agents in infectious diseases of mammals caused by bacteria, especially diseases like tuberculosis (TB) and leprosy caused by \textit{mycobacteria}. Thiazinone and their derivatives are used as antibacterial agents, especially against \textit{mycobacteria} (TB) [58].

Michael \textit{et al.} have published their findings on \textit{M. tuberculosis} porins and toxins. \textit{M. tuberculosis} infection has been supposed to engulf about two billion people and the lung of a single infected patient is known to contain more than a billion \textit{bacilli}. Poor treatment compliance resulted in increasing spread of multi- and extremely- drug resistant strains. This paper related to the isolated polypeptides comprising the amino-terminal domain of \textit{M. tuberculosis} porin A (MtpA), wherein the polypeptide was a porin monomer. It also talked about isolated polypeptides comprising the carboxyterminal domain of \textit{M. tuberculosis} porin A and a second polypeptide comprising an antigen and the use the chimeric porin polypeptides in methods of eliciting an immune response in a subject [59].

Chopra \textit{et al.} have invented compounds such as Benzotriazine oxides as a drug targeting \textit{Mycobacterium tuberculosis} whose structure formula comprises of X which is independent, H, halogen, alkyl, OR, SR, NR’R, BR’R, heterocyclic, or another functional group, where each R is independent H, halogen, alkyl, OR, SR, NR’R, BR’R, heterocyclic, or another functional group; W is N, C, O, S, H or B or another linking atom; each A and B is H or optionally substituted alkyl, which may be joined in an optionally as hetero- or cycloalkyl; and Z is an optionally present, optionally substituted 4-8 membered ring, saturated or unsaturated, fused to the benzotriazine ring at either the 6,7-, 5,6- or 7,8-position, or a pharmaceutically accepted as salt or stereoisomer [60].

Skeiky \textit{et al.} have provided polypeptides and fusion proteins, which comprise of a mutated version of MTB32A, in which amino acids such as histidine, aspartate, or serine at the active site had been mutated to a different amino acid. Also fusion polynucleotides, fusion polypeptides, or compositions that contain at least two heterologous \textit{M. tuberculosis}-coding sequences or antigens were highly antigenic and upon administration to a patient, increase the sensitivity of \textit{tuberculosis sera}. Thus, the invention comprise dof isolated or purified \textit{M. tuberculosis} polypeptides and nucleic acids which could be used as pharmaceutical compositions for administration into a subject for the prevention or also for the treatment of \textit{M. tuberculosis} infection. The immunogenicity of the fusion protein or antigens may be enhanced by the inclusion of an adjuvant and additional fusion poly-
peptides, from *Mycobacterium* or other organisms, such as bacterial, viral, mammalian polypeptides [61].

Meyer et al. have described the use of a naphthoquinone derivative in pharmaceutically acceptable salts and in the manufacture of a medicine to be used in a method for treating as well as controlling tuberculosis in a patient caused by *Mycobacterium tuberculosis*. Diospyrin and methyljuglone, naphthoquinone derivatives of naphthoquinone from *E. natalensis* and other species in this genus, as well as in other plant species that may synthesisediospyrin or methyljuglone or other quinone derivatives have been found to inhibit several antibiotic resistant as well as antibiotic susceptible strains of *M. tuberculosis* [62].

The invention of Brickner et al. of a method for treating tuberculosis in a mammal involved the administration to said mammal an effective amount of a compound -(S)-N- [[3-[3-fluoro-4-(4-thiomorpholinyl) phenyl]-2-oxo-5-oxazolidinyl] methyl] acetamide, or a pharmaceutically acceptable salt in combination with at least two agents used in the treatment of tuberculosis along with the therapeutically effective amount of at least one agent and also at least one pharmaceutically accepted carriers [63].

As the interaction with the single of strain of *M. tuberculosis* remains to prevail, TB remains to be the most life threatening disease across the globe, infecting one third of the world population latently. The current procedure of treatment, involved a mix of first and second line drugs, takes roughly up to nine to six months. The paper by Riccardi et al. threw some light on the need to discover new drugs, and hence mentioned the discovery of the protein Rv3790 (from *M. tuberculosis*), the target for benzothiazinone drugs, a new class of molecules that appear to be very promising in the treatment of tuberculosis [64].

Protopopova et al. have found methods and compositions comprising substituted ethylene diamines for the treatment of tuberculosis. This was achieved by preparing a chemical library of substituted ethylene diamines, which allows for the synthesis of a diverse set of substituted ethylene diamines. These diamines are screened for anti-TB activity using in vitro, biological assays, including a High-Throughput Screening (HTS) assay [65].

**CONCLUSIONS**

TB in its new and aggressive form cuts across sections of society, threatening anyone with a weakened immune system, most commonly targeting lungs (pulmonary TB); also affecting other organs including the brain, the kidneys and spine (in case of extra pulmonary TB). By analyzing various patents on several signaling pathways, pathways such as NFkB signaling, lipid metabolism, TCA cycle and glycolate lute shunt have been studied and patented encompassing the diagnostic and therapeutic applications of anti-tuberculosis remedial treatment. Conventional TB diagnosis continues to rely on sputum smear microscopy, culture, tuberculin skin test, and chest radiography. It is essential that effective antibacterial agents are administered in appropriate amounts in order to treat the condition of Tuberculosis, hence important drugs like INH, RIF, PZA and their workings have been briefly discussed.

**FUTURE PROSPECTS AND CHALLENGES**

The fight against TB is not over yet, and it seems as if it is here to stay for a little while longer. Though the current lines of both detection and treatment employ several techniques like smear microscopy, nucleic acid amplification tests etc. and drugs like Isoniazid, Rifampicin, Pyrazinamide etc., but the ever increasing prevalence of multi drug resistance in MTB calls for new and improved methods for diagnosis and treatment of the same. The use of expanded clinical case definitions of smear negative TB, with help of little further research, has shown to have a promising future. It involves the assessment of the sample (either induced sputum or blood or urine) by means of symptom review, cultures or chest X-rays; followed by abdominal and pericardial ultrasonography for patients with normal chest X-rays. Similarly, Microscopic Observation Drug Susceptibility assay (MODS), a culture Though the current lines of both detection and treatment employ several techniques like smear microscopy, nucleic acid amplification tests etc. and drugs like Isoniazid, Rifampicin, Pyrazinamide etc., but the ever increasing prevalence of multi drug resistance in MTB calls for new and improved methods for diagnosis and treatment of the same. The use of expanded clinical case definitions of smear negative TB, with help of little further research, has shown to have a promising future. It involves the assessment of the sample (either induced sputum or blood or urine) by means method involving direct observation of *Mycobacterium tuberculosis* and simultaneous yielding of drug-resistance, promises a much more sensitive, faster and cheaper detection of TB than current culture based tests. High throughput screening of proteins in bodily fluids using proteomics or DNA microscopy to define biomarkers of either the host or the microbe is an approach that stipulates a more sensitive and less time consuming approach to diagnose TB. Since most of the MTB strains are known to develop resistance to drugs over a period of time, it is unlikely for the pharmaceutical industries to generate enough revenue to cover the costs of developing novel drugs against Tuberculosis; hence not many new antibacterial agents against TB have emerged in the last few decades. But recently, new approaches to developing therapy against TB sure have come in light. These approaches need the modification of existing classes of drugs for greater specificity; evaluation of new classes of drugs; and new modes of drug delivery into patients’ systems. Some drugs that under pre-clinical treatment, namely, PA-824 (Nitroimidazolone), KQR-10018 (Quinolizine) and SQ109 (Ethinambutol analogue) are stipulated to play a potent role against TB in future. Their working mechanisms and effects on the host are still not very clear, and need a little bit more of research. A major challenge will be to sustain and increase funding for continued developmental and clinical work if the promise of tuberculosis elimination, or at least significant lessening of the global tuberculosis epidemic, is to be achieved.
CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>MTB</td>
<td><em>Mycobacterium tuberculosis</em></td>
</tr>
<tr>
<td>BAC</td>
<td>Bacterial Artificial Chromosome</td>
</tr>
<tr>
<td>APC</td>
<td>Antigen Presenting Cells</td>
</tr>
<tr>
<td>NFkB</td>
<td>Nuclear Factor Kappa light chain enhancer of activated B cell</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumour Necrosis Factor</td>
</tr>
<tr>
<td>TCA</td>
<td>TriCarboxylic Acid cycle</td>
</tr>
<tr>
<td>CMI</td>
<td>Cell Mediated Immunity</td>
</tr>
<tr>
<td>NAD</td>
<td>Nicotine Adenine Dinucleotide</td>
</tr>
<tr>
<td>INH</td>
<td>Isoniazid</td>
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<td>RIF</td>
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REFERENCES


