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NEW INSIGHTS INTO LATENT TUBERCULOSIS: A PERSISTING GLOBAL HEALTH CHALLENGE

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ABSTRACT

After more than a century, tuberculosis (TB) is still a formidable public health challenge as it contributes considerably to illness and death worldwide, irrespective of continuous advancement in technology and in depth understanding of molecular biology concepts. *Mycobacterium tuberculosis* persist for longer period in human and animal host in latent state without causing active disease and may reactivate soon after immunity declines. Today's chemotherapy is lengthy as well as ineffective to control tuberculosis. Moreover, in the absence of fundamental biological understanding of mycobacterial persistence, significant pathogen driven factors and validated animal models, shortening the duration of anti-tuberculosis therapy remains a distant goal. Subsequently, molecular signaling proteins which serve as potential targets should be explored and explicated to limit mycobacterial growth. This review focuses on mechanisms of mycobacterial persistence, various models of latent tuberculosis, identification of crucial gene targets, vaccination and therapeutic approaches to target latent tuberculosis infection.

INTRODUCTION: Tuberculosis (TB) continues to be one of the most significant infectious diseases in terms of human morbidity and mortality**.** *Mycobacterium tuberculosis* (*M. tuberculosis*) is a multifaceted pathogen responsible for acute as well as latent tuberculosis infection (LTBI). LTBI is an asymptomatic subclinical condition containing viable and metabolically silent *M. tuberculosis* bacilli. Infected individuals who exhibit significant immune response against these antigens may suffer from active TB at later stages of life [1]. According to World Health Organization (WHO), about one third of the world's population is infected with LTBI with mortality rate of about 2 million each year. WHO estimated 9.4 million incident cases of TB worldwide in 2009, a slight increase from 9.27 million in 2007, of which South-East Asia Region accounting for 35% of total incident cases. However, the estimated incidence rate in sub-Saharan Africa is nearly twice that of the South-East Asia Region with over 350 cases per 100000 populations. In 2008, the estimated per capita TB incidence was stable or falling in all the six WHO regions.

However, the slow decline in incidence rates per capita is offset by population growth. Consequently, the number of new cases arising each year is still increasing globally in the regions of Africa, the Eastern Mediterranean and South-East Asia. An estimated 1.7 million people died from TB in 2009. The highest number of deaths was in the African Region (50 per 100 000 population). As per WHO estimates, there were 13.6 million prevalent cases of TB in 2009 (164

per 100 000 population), a slight decrease from 13.7 million cases (206 per 100 000 population) in 2007. Prevalence and mortality rates are falling globally in all the six WHO regions. The Region of the Americas as well as the Eastern Mediterranean and South-East Asia regions are on track to achieve the 'Stop TB Partnership' targets of reducing prevalence and death rates by 50% by 2015, compared with a baseline of 1990 [1].

Preliminary diagnosis of LTBI involves tuberculin skin test (TST), in which purified protein derivatives (PPD) of *M. tuberculosis* are administered intracutaneously to suspected individuals resulting in local delayed type hypersensitivity reaction. Diameter of induration is measured within 48-72 h. In duration of ≤5mm is considered positive in HIV infected persons, ≤10mm in injection drug users or recent immigrants from high prevalence area and ≥ 15mm in low risk TB patients [2].

Despite its wide use, TST suffers from inherent limitation such as inter–observer variability, false positive results in case of BCG immunized individuals, false negatives in case of immunocompromised patients with low T-cells count. Therefore, TST results have to be interpreted taking into consideration the pretest risk of TB or its reactivation. To overcome these problems, recently T-cell interferon-gamma release assays (IGRA) have been introduced which involve diagnostic techniques like QuantiFERON-TB Gold assay and T-SPOT TB test. These are based on specific antigens viz. 6kDa early secreted antigenic target (ESAT-6) and culture filtrate protein (CFP-10).

Moreover, these tests are more specific and probably more sensitive than TST which measures secretion of interferon-gamma by T-cells stimulated in response to antigens and thus potentially represent a significant advancement in the diagnosis of LTBI. A positive IGRA only indicates a long-lasting T-cell immune response against *M. tuberculosis* unlike TST it does not demonstrate the persistence of viable bacilli [3, 4]. Emphasizing on association of TB and HIV co-infection, at least one-third of the 42 million people living with HIV worldwide are infected with TB. These patients have much higher risk of reactivation of TB (estimated at 10% per year) as compared to normal population (estimated at 10% in a lifetime).

The treatment of LTBI significantly reduces chances of reactivation. Hence, identifying and treating HIV infected persons for LTBI is a high priority area. In addition to above co-infection, other risk factors responsible for progression to active TB include silicosis, diabetes mellitus, chronic renal failure, cancer of the head, neck, and lungs, malnutrition, weight loss, leukemia, lymphoma, and gastrointestinal surgery [5]. Modern multidrug chemotherapy is effective against active TB which eradicates tubercle bacilli from its primary reservoir but unable to fight against latent bacilli due to their slow replication rate and low burden in infected host.

Such therapy is based on the assumption that patients follow standard anti-TB treatment regimen for 9-12 months. Improper diagnosis of LTBI due to a variety of reasons may lead to reactivation of active disease, making its global eradication even more difficult than the already ambitious goals embraced by WHO [6]. A person infected with LTBI usually shows exceptional asymptomatic characteristics viz. a positive TST or blood test, a normal chest X-ray, a negative sputum test, viable but inactive TB bacilli and no feeling of sickness.

How bacilli turn up into latent state? During normal course of infection *M. tuberculosis* exists in active, latent, dormant or persistence phases. Due to vigorous cell mediated immune response(s), progress of infection gets arrested resulting into formation of Ghon complex i.e. scars containing calcified lesions in lung parenchyma and local lymph nodes which represents a quiescent state indicating establishment of LTBI. After few years, latent bacilli may reactivate, leading to active TB depending upon immune status of infected host [7].

On the contrary, dormancy means metabolically silent state of TB pathogen. TB lesions are said to be either active or dormant, depending upon the nature of associated pathology either progressing or healing. Active lesions comprise of acid fast bacilli, however bacteriological status of dormant lesions is still unknown [8]. TB bacilli are capable of persisting slowly in infected host for many years without causing symptomatic disease.

Henceforth, mycobacterial persistence is and continues to be a major threat responsible for lengthy chemotherapy. This bacillary persistence can be deciphered by either arresting bacillary growth or spontaneous resolution of TB [9]. Some studies have shown that latent bacilli are neither acid fast nor enough in number to be detected by conventional microscopic techniques which is absolutely contrary to active TB.

During latency, bacilli are harbored within the granulomatous lesions or pulmonary lymph nodes of infected host. But this explanation is unable to prove the presence of extrapulmonary TB which accounts for 15% of the total cases. In extrapulmonary TB, reactivation originates from bones, brain, lymph nodes or other sites. These findings suggest an alternative hypothesis that latent bacilli not only reside in pulmonary sites but also disseminate throughout the body triggering a widespread LTBI [10].

Host innate immune responses in latency: TB infection starts when bacilli reside and replicate within alveolar macrophages. At the same time, dendritic cells (DC) and monocytes get activated which induces naïve and memory T-cells. Their role is important in initiation and maintenance of protective immunity. Pathogen is able to survive within the cell due to its ability to prevent phagosome acidification as well as phagosome-lysosome fusion. It is rarely eliminated from host, suggesting that human beings represent a large reservoir of mycobacterial population [11, 12].

Macrophages and lymphocytes migrate to the site of infection and form granuloma which act as protective structures limiting the spread of pathogen. Granuloma consists of epitheloid macrophages, DCs, T-cells, B-cells and fibroblasts [13]. Macrophages and DCs recognize the conserved molecular patterns expressed on TB bacilli with the help of toll-like receptors (TLRs) [14, 15]. Binding of TLRs with pathogen-specific ligands activate signal transduction pathway in host T-cells resulting in activation of NF-κb and induction of cytokines such as IL-12 and chemokines that are essential for eliciting an adaptive immune response [16-18]. Many studies have been performed to investigate the exact role of DCs and macrophages in pathophysiology of TB.

Usually DCs activation results in arrest of *M. tuberculosis* growth whereas macrophages exhibit bactericidal effect. Some studies have suggested the role of DC as a reservoir for tubercle bacilli in tissues including lymph nodes and lungs [19, 20].

T-cells are responsible for activation and destruction of infected macrophages. In HIV infected population, decay of $CD4^+$ T-cells increases the chances of TB reactivation [21]. Some investigators have shown that mice deficit of $CD4^+$ T-cells were more prone to fatal TB leading to death [22]. Adoptive transfer of $CD4^+$ T-cells has been proved to protect these mice against TB. It has been assumed that $CD4^+$ cells are produced in early stages of infection whereas $CD8^+$ cells controls later stages as detected by their presence in granuloma [23].

Some evidences show that nitric oxide synthase induces production of Reactive Nitrogen Intermediate (RNI) [24]. The host has capability to control mycobacterial replication by means of Th1 immunity with concomitant production of IFN-γ and subsequent formation of lungs granulomas which further activate macrophages leading to production of RNI. This is the most accepted mechanism by which macrophages can kill intracellular *M. tuberculosis* [7, 24]. However, some other additional mechanisms have also been proposed to support the role of IFN-γ in TB pathophysiology. This can be depicted from a study in which mice deficient of IFN-γ or its receptors were more susceptible towards TB as compared to those deficient in NOS2 due to its inability to produce RNI. An analogous picture also exist in humans which show enhanced susceptibility to TB [25-27].

TNF-α is a vital cytokine required to control acute as well as LTBI. TNF-α deficient mice have been shown to have decreased granuloma formation and concurrent increase in bacterial count resulting in immediate death. Moreover, they exhibit an altered chemokines expression pattern indicating TNF-α has capability to regulate chemokines gradient and subsequent recruitment of cells into granuloma. On the other hand, another study showed an intense inflammation in mice resulting in keratin deposition and squamous metaplasia without significant necrosis [28].

Earlier studies in murine model have shown that TNF-α is critically required in defense mechanism as mice treated with anti-TNF-α-antibody become more susceptible to BCG infection [29, 30]. During infection, amount of TNF-α in lungs solely determines whether cytokine is protective or destructive [31]. TNF-α receptors (TNFαR) deficient mice failed to control bacterial replication resulting in increased susceptibility to TB. Also, it has been demonstrated that the use of TNF-α blockers in chronic inflammatory diseases (like rheumatoid arthritis and Crohn's disease) result in TB reactivation [32, 33].

Both TNF-α and IFN-γ play important role in innate immunity by inducing macrophages activation, RNI production, granuloma formation, and subsequent killing of pathogen. A study conducted in murine model showed a continuous activation of macrophages due to high level of various cytokines [34, 35]. Some studies indicated that inhibition of NOS2 activity results in reactivation of TB due to sharp rise in bacterial count in mouse lungs without any significant effect on liver and spleen counts [36]. On the contrary, the organs of mice deficit of NOS2 or treated with NOS2 inhibitors showed a significant increase in mycobacterial count [24].

Models of LTBI: Due to poor understanding of relationship between critical pathogen driven factors and host immune response in LTBI, there is an urgent need to establish a perfect experimental system which can serve as a model. To evaluate critical aspects of immunopathology of LTBI various models are studied and discussed as follows.

A) ***In vitro* models:** Evaluation of new chemical entities (NCEs) against latent state *M. tuberculosis* requires appropriate *in vitro* models. In the past, following models were suggested such as cultivation at 8°C for 31 days, nutrient starvation, oxygen depletion in vigorously shaken broth cultures, anaerobic model in submerged broth cultures and cultivation of cultures at pH 4.8–5.0 in broth for 6 weeks. Amongst these models, transient oxygen depletion and nutrient starvation induced models are studied in detail and most widely used for screening of potential antitubercular agents.

i. **Hypoxia induced model:** Latency is assumed to be associated with hypoxic conditions within the infected host. In Wayne model, *M. tuberculosis* undergoes a hypoxia induced non-replicating persistent (NRP) state of metabolism. This model is based on gradual adaptation of *M. tuberculosis* to microaerophilic conditions and ultimately leading to anaerobic state as a result of metabolic alterations at cellular level [37]. Hypoxic shock for a defined period results in shift down of bacterial respiration to lower oxygen levels involving nitrate reduction and subsequent induction of metabolic, chromosomal and structural changes in bacilli [38].

Genomic and proteomic expression studies have shown upregulation of several important proteins including lysine dehydrogenase and nitrate reductase [39]. Under such conditions, bacilli remain viable and are capable of replicating in synchronous fashion until return to oxygenated media. They do not divide or synthesize DNA and become resistant to first line chemotherapeutic agents such as isoniazid, rifampin, pyrazinamide etc. On the other hand, they are sensitive to nitroimidazole such as metronidazole under *in vitro* hypoxic conditions [40, 41].

Unfortunately, metronidazole fails to exhibit suitable antitubercular activity in an *in vivo* hypoxic granuloma model [42]. Nonetheless, a novel nitroimidazopyran compound i.e. PA-824 has shown better bactericidal activity against *M. tuberculosis* under both aerobic as well as anaerobic conditions *in vitro* and murine models of LTBI. These findings substantially increases the scope of development of PA-824 as a new option for therapy [43].

ii. **Nutrient starvation induced model:** Apart from hypoxic shock, it is also assumed that tubercle bacilli may undergo nutrient starvation under latent state. These starved bacilli adapt to increase their tolerance toward adverse environmental stresses and exhibit a low respiration rate, yet maintain long term viability. The relatively large number of differentially

expressed genes indicates that nutrient starvation conditions induce a global shift in gene expression profile of *M. tuberculosis* [44]. Furthermore, there is a down regulation of few genes that are key component of amino acid biosynthesis, DNA replication, repair and restriction/modification, biosynthesis of cofactors/prosthetic groups and carriers, energy metabolism, lipid biosynthesis, translation and post-translational modification [45].

On the other side, there is up-regulation of genes such as isocitrate lyase (icl), an enzyme essential for fatty acid metabolism which facilitates mycobacterial survival in NRP state. It has been proved that disruption of icl gene attenuates bacterial persistence and virulence in immuno-competent mice without affecting bacterial growth during the acute phase of infection [46].

B) ***In vivo* models:** Mouse, guinea pig, rabbit and non-human primate (NHP) are some of the animal models of TB. In order to evaluate anti-TB activity of different drugs or NCE, it is very important to develop and establish an animal model of LTBI.

i. Guinea pig: Although guinea pigs are considered one of the best animal models of TB, little data supports their utility in LTBI. However, some of the new research findings suggested that lung lesions in guinea pigs infected with *M. tuberculosis* have striking similarities, such as necrosis, mineralization, and hypoxia to that of human infection. Guinea pigs can easily develop primary and secondary necrotic lesions. Conventional therapy for tuberculosis for specified periods reduces the bacterial load significantly without any considerable effect on primary tubercular granulomas [47]. Moreover, some studies claims the presence of latent bacilli in guinea pig lungs and spleen and shown a positive TST test. All these findings suggest guinea pigs may be used as a reliable animal model of LTBI [48].

ii. Mouse model: Relatively, resistant nature of mice to acquire TB infection has been utilized for easy development of suitable model of LTBI. Mouse model replicates many facets of human immune response, however they fail to demonstrate formation of granulomas; lack caseous and necrotic centers. The intrinsic advantages of this model include its cost, size and availability, the vast potential for manipulation including genetically modified strains [49]. Two murine models of LTBI are widely used, one of these, Cornell model was developed by McCune and colleagues at Cornell University in 1950s, where mice were infected with high dose of *M. tuberculosis* and treated with isoniazid and pyrazinamide for 3 months, after which no culturable bacilli were recovered.

However, after cessation of chemotherapy, one third of mice relapsed and almost all the mice relapsed after administration of immunosuppressant steroids. These recovered bacilli were fully susceptible to above drugs indicating that dormant bacilli are only phenotypically resistant [50]. In this model, metronidazole has been found to be ineffective in preventing reactivation [51]. However, it has bactericidal potential against latent *M. tuberculosis* grown under *in vitro* hypoxia conditions [40].

Different researchers have also used a modified Cornell model which was developed by Flynn *et al*, to study LTBI in infected host. This model involves modulation of inoculum, duration of antibiotic therapy, antibiotic dosages, and time interval between cessation of antibiotics and immunologic interventions [52]. This model detects very less number of bacilli and these levels could be maintained for many weeks. On the contrary, it is an artificial model showing less stability and poses spontaneous reactivation either with senescence or accelerated by immunosuppressive therapy. This model needs complete standardization.

This model does not mimic human LTBI because latency is attained with chemotherapy [53, 54]. Despite above limitations, it has been used in numerous studies to understand host response to different pathogenic strains and mutants.

iii. **Non-human primate model:** NHP models are preferred amongst all these models due to their close resemblance with human infection. Macaque model is used for LTBI. Cynomolgus macaques (Macacca fascicularis) infected with low dose of *M. tuberculosis* result in spectrum of disease that mimics the human infection. Studies have confirmed that about 65-70% of infected NHPs develop LTBI which is illustrated from positive TST test and lymphocyte proliferation assay using specific mycobacterial antigens [55]. Necropsy findings explain enlargement of hilar lymph nodes with or without granulomas. These granulomas are often fibrotic and may be calcified. Cavitary lesions with liquefaction of caseum may occur as in humans. Evidences indicate that NHPs are the best model for studying immunology and pathology of TB along with pharmacological effects of drugs and vaccines [56].

iv. **Rabbit:** *M. tuberculosis* infected rabbits show extreme resistance to infection which may result in the development of LTBI. CFU counts from lungs usually increases sharply within 4 weeks of infection, but after 4 months animals show negative culture. Interestingly, after 6 months, some animals show culturable colonies, suggesting existence of bacillary persistence at very low level [50]. Also, some of the studies with M. bovis have shown close similarities with human infection with formation of caseum and cavities. A recent rabbit aerosol infection model developed by group of researchers has shown occurrence of bacillary replication for about 5 weeks with caseous necrosis followed by gradual decrease in bacillary count over the period of 36 weeks. The rabbits were TST positive as well as clinically asymptomatic. This model does not suffer from spontaneous reactivation [57].

C) ***In silico* models:** Mathematical modeling of biological systems involves qualitative and quantitative formulation of models, their translation into efficient computer implementations, designs of appropriate algorithms, estimation of parameter values, output visualization, and comparison of simulation results for further experimentation. Drug development and testing is also accessible besides prediction of host-pathogen interaction along with key aspects of biology (such as IFN-γ, TNF-α and IL-12), as an important step towards validation [58]. These models have predicted several features that require experimental testing against TB. For example, IL-10 knockout mice infected with *M. tuberculosis* have shown no phenotypic differences as compared with wild type mice. However, virtual models of human LTBI simulating IL-10 knockout elucidated high chances of reactivation in the absence of IL-10 [59].

Gene targets against latent state *M. tuberculosis*: In order to understand the mechanism by which *M. tuberculosis* regulates its different genes to adapt to environmental changes will lead to comprehension of many interesting aspects of latency and host-adaptation. The availability of genome sequences and development of microarray-based comparative genomic analysis has lead to the publication of several virulence factors and gene expression patterns under simulated latency conditions.

Absence of selective and well-defined targets has been a major obstacle in the development of effective chemotherapy for LTBI [60, 61]. Biomarkers which segregate patients by diagnosis, prognosis and appropriate therapeutic selection criteria are needed and will form the basis of prospective clinical management. Limited success has been achieved in LTBI biomarkers discovery. The stepwise process used to generate these therapeutic targets is illustrated in **Fig. 1**.

In LTBI gene targets that show both upregulation as well as mycobacterial growth inhibition are certainly more interesting, but some of them may not affect growth pattern. It is well-established that the genes expressed under hypoxic or nutrient starvation conditions are not critical for intracellular growth in macrophages and some of them are discussed below [62].

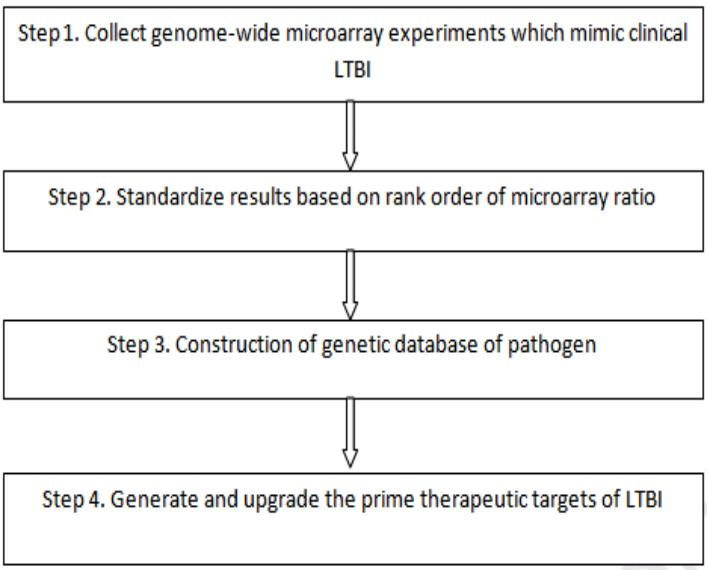

FIG. 1: FLOWCHART OF STEPWISE PROCESS USED TO GENERATE LIST OF THERAPEUTIC TARGETS

i. **DevR/ DosR regulon:** Experimental evidence has shown that transcriptional regulator DevR (viz. DosR), controlling a 48-gene regulon, is a critical factor involved in mycobacterial persistence. DevR is necessary for the induction of a robust genetic response to reduce oxygen tension [63]. It has been observed that nitric oxide activates devS and DevR resulting in metabolic shift down and finally lead to LTBI. Without nitric oxide signal, regulon fails to up-regulate and cell division continues until the host is killed. DosR is responsible for regulation of many genes, however its role for some of the induced genes is unclear [64].

ii. **Universal stress proteins:** Universal stress proteins **(**USP) are a family of proteins that are produced by mycobacteria in response to variety of environmental stressors but their exact role in TB pathogenesis is unclear. A recent study by Drumm et al suggested that USP homolog, Rv2623, binds to ATP in nucleotide binding pockets. This is essential for entry of bacilli into chronic persistent growth phase and further regulates mycobacterial growth *in vitro* and *in vivo* [65]. These findings provide valuable insight into LTBI and reveal new opportunities for the development of novel anti-TB therapies.

iii. **Carbon metabolism:** The dual stress of unbalanced nutrient pool and hypoxia poses significant demands on regulation of metabolites through two-carbon pathways. Transcriptional analyses suggest that *M. tuberculosis* survives in the host on a nutrient pool rich in fatty acids and poor in carbohydrates [66]. Fatty acids (triacyl glycerol) are important nutrient source for these bacilli possessing about 250 lipid metabolizing enzymes. In carbon metabolism, genes responsible for hypoxic environment in granuloma are significantly upregulated.

iv. **Pantothenate biosynthesis:** Acetyl-coenzyme A (Ac-CoA) is a central intermediate in primary metabolic activity of *M. tuberculosis* which plays roles in TCA cycle as well as fatty acid and amino acid biosynthesis. The flux of carbon is particularly critical for survival of latent *M. tuberculosis* via fatty acids breakdown and glyoxylate shunt. The mycobacterial membrane is critically dependent on this carbon flux for maintaining its integrity [67, 68].

v. **Downregulation of ATP synthesis:** *M. tuberculosis* is assumed to undergo inadequate ATP synthesis in NRP state; hence ATP synthesis is likely to be a promising strategy to control LTBI. The newly disco0vered diarylyquinoline, an inhibitor of ATP synthase proton pump, may serve as a valuable tool for evaluating this approach [69]. Compounds with similar mode of action such as 2, 4-dinitrophenol may work if they are designed selectively. Moreover, strategy of depleting ATP stores may be effective towards targeting LTBI [64].

vi. **Downregulation of protein synthesis:** In LTBI, both protein synthesis and replication declines sharply. This is confirmed from data indicating significant down-regulation of many genes involving 30S and 50S ribosomal proteins. Rifampin is one of the few effective drugs active against tubercle bacilli in NRP-2 phase [70]. It has shown efficacy against latent *M. tuberculosis* by protein synthesis inhibition. Thus proteins synthesis inhibitors could be amongst the possible chemotherapeutic agents [71].

vii. **Chaperonins/HSP:** Chaperonins or heat shock proteins (HSP) have stabilizing effect on *M. tuberculosis* resulting in reduction in effectiveness of rifampin against LTBI. Thus it is hypothesized that a combination of rifampin and chaperonin inhibitor may shorten therapeutic regimen of LTBI.

During latency, acr gene is up-regulated that encodes an α-crystallin homolog, usually recognized by immune response in infected host [72]. Recent work has shown more rapid initial growth and increased CFU counts in mouse lungs and spleen in acr1 knockouts. These findings suggest that acr1 has vital role in mycobacterial growth rate during initial stages as well as long term persistence [23, 73].

Vaccine strategies to target LTBI: Increasing global health impact of TB and HIV has already worsened human life on the earth. Today's anti-TB therapy is not completely effective to eradicate TB from infected population. At the same time, emergence of MDR-TB and LTBI has become complex and diverse challenge which necessitate an urgent need to develop potential chemotherapeutic agents along with vaccination approach. Before design of effective vaccines, current research activity should focus on solving three key challenges

- Identifying the specific types of immune responses induced by effective vaccines
- Identifying effective new antigens for eliciting such immune response(s)
- Improving reliability of animal tests used to evaluate potential vaccines prior to human trials

The conventional BCG vaccine was introduced about 60 years ago which has been accepted worldwide to prevent TB amongst the children. Also, it gives protection against leprosy and tubercular meningitis. Although a single dose of BCG confers about 70% immunity, the level of protection varies considerably [74, 75]. About 18 years ago, WHO has declared directly observed treatment-short course (DOTS) as the global strategy to control TB. Unfortunately, this strategy is inadequate to control global TB epidemic.

Most of the vaccines are designed as prophylactic vaccines in order to limit infection and boost immunity. Current strategies suggest that prophylactic vaccines could be designed either as live mycobacterial vaccines to replace BCG or subunit vaccines to boost immunity. It is necessary to develop novel post-exposure vaccine which targets latent stage of infection and prevent reactivation in individuals living in TB endemic areas [76, 77]. Prevention is always better than cure and vaccination can serve as an optimal approach for TB control program. Ideally, new vaccines with high profile of safety and efficacy are needed to provide greater level of protection as compared to BCG. Current research activity in TB vaccination is aimed at designing and developing novel vaccines either prophylactic or post-exposure or combination of both. Some of these are on their way to preclinical and many in the clinical stages of development (listed in **Table 1**) [78].

TABLE 1: NEW ANTI-TB VACCINE IN PIPELINE OF CLINICAL TRIAL

Vaccine Name	Vaccine Type	Developmental stage	References
rBCG30	Live, recombinant BCG	Phase I completed in 2004	79
rbcg::ureC-llo+	Live, recombinant BCG	Phase I completed in 2007	80
MVA-85A	Modified vaccinia virus	Ongoing clinical phase I	81
H1/IC31	Adjuvanted subunit	Ongoing clinical phase I	82
Mtb72f	Adjuvanted subunit	Ongoing clinical phase I	83
H1/LTK63	Adjuvanted subunit	Phase I completed in 2007	84
HyVac4/IC31	Adjuvanted subunit	Phase I completed in 2007	85

Generally, prophylactic vaccines are designed on the basis of early antigens like ESAT-6, Ag85 and others recognized by host immune system during initial stage of infection. No doubt, these vaccines are quite better than BCG, but none of them showed any significant sterilizing immunity. Earlier preclinical research has demonstrated the capability of BCG to control mycobacterial growth, however it fails to prevent infection in comparison with non-vaccinated animals [86]. In similar pattern, even though large number of global population is BCG vaccinated yet they harbors latent bacilli and out of this 5-10% of cases lead to reactivation. However, some research findings have suggested slight induction of immune response(s) against some of the late stage antigens in BCG vaccinated individuals. This could be one of the reasons for failure of BCG vaccine against LTBI [77, 87-89].

Prophylactic vaccines: Currently available TB vaccines are generally prophylactic vaccines. Unfortunately, these vaccines are unlikely to be effective in individuals already infected with *M. tuberculosis*. Some proteins of Rpf family are assumed to have fulfilled the purpose of prophylactic vaccines. Out of these, five proteins were found to be immunogenic and efficacious in mouse model [90]. Also, it has been assessed that Rv3407, a gene of Rpf proteins family, serves as DNA vaccine which gives significant protection against *M. tuberculosis*. Unfortunately, these vaccines have not been further checked for their activity in already infected subjects [91].

Post-exposure vaccines: This approach has been a subject of debate due to safety concerns and their role in providing protective immunity. Vaccination studies on mouse model confirmed that deleterious Koch like reaction that are usually observed in latently infected immunized individuals are quite low [92]. On the other hand, latently infected population usually has a high level of T-cell response against mycobacterial antigens. Hence, the requirement of these vaccines to boost immune response in already vaccinated individual is not completely understood [93, 94]. Beside this, such vaccines are able to target and redirect human immune response to late stage antigens which help in prevention of reactivation or relapse of disease.

Treatment of LTBI: Although TB kills two million people a year (one person every 15 seconds), there is no new drugs approved to treat TB in last 40 years. According to WHO, the global incidence of TB is rising by 1% per annum [94]. The increasing impact of synergism between TB and HIV, as well as the emergence of MDR cases pose a serious attention to develop and adopt new anti-TB strategies to eradicate this harmful pathogen from human beings [95].

Most of research effort in TB drug development deals with early stages of drug discovery including basic research aimed at identifying and validating drug targets and screening lead compounds. But few leads are being optimized to generate prospective drug candidates [96]. Several strategies are being pursued in order to identify new leads. These include making derivatives of existing drugs and screening their activities in an *in vitro* cultured whole cells, isolated essential targets, *in vitro* models mimicking persistence and targets required for survival in humans [61].

Identifying and treating LTBI is a key component of global efforts to eliminate TB. Unfortunately, treatment usually requires prolong course of antibiotic therapy. The current drug regimen used to treat LTBI is summarized in Table 2.

TABLE 2: DRUG REGIMEN FOR LTBI

Drug regimen	Duration	Administration	Effectiveness	Toxicity
Isoniazid	9 months	daily or twice-weekly	75%-85%	Lower hepatotoxicity &/or peripheral neuropathy
Rifampin	4 months	daily self-administered	65%-75%	lower than isoniazid
Isoniazid + rifampin	3 months	daily self-administered	55%-60%	lower than isoniazid
Isoniazid + rifapentine	3 months	once weekly	60%-70%	lower than isoniazid
Rifampin + Pyrazinamide	2 months	daily self-administered	70%-80%	severe hepatotoxicity

The preferred regimen of treatment is isoniazid for 9 months or 270 doses for 12 months. TB reactivation can be greatly diminished by 6 to 12 months course of isoniazid alone, which is 75% ~ 93% efficacious. As it is evident from the fact that isoniazid is effective only against actively dividing TB bacilli, recent data has suggested that isoniazid might be effective in cases where dormant bacilli attempts to divide [97]. Several surveillance studies confirmed that isoniazid associated hepatotoxicity result in increased mortality along with aging.

In the United States, it is currently recommended that patients on isoniazid must undergo monthly clinical assessments for adverse effects and consulted properly for symptoms [98]. Another 4 months drug regimen of rifampin is very useful for the patients either intolerant or resistant to isoniazid. Rifampin appears to be comparatively safe, well tolerated and is quite selective towards eliminating active TB. However, its use should be avoided in patients suffering from HIV co-infection due to high risk of drug resistance.

Drug-drug interactions between these two therapies have already caused crisis, as rifampicin causes cytochrome P450 isoenzyme induction which drastically lowers plasma concentrations of HIV protease inhibitors.

Thus, care should be taken while prescribing such cocktail therapy and the future research should be oriented towards avoiding such incompatibilities between anti-TB and anti-HIV therapeutic agents [99]. Apart from monotherapy, some researchers have developed combinational drug approach to treat LTBI. Out of these, isoniazid plus rifampin for 3 months regimen is about 55%-65% efficacious in preventing reactivation of disease as compared to placebo. Moreover, this combination is equally safe in 6 to 12 months of isoniazid therapy.

Secondly, 3-months regimen of once-weekly isoniazid plus rifapentine is getting importance in clinical trials because of long half life of rifapentine and low toxicity. The American Thoracic Society and the US Centers for Disease Control and Prevention issued revised treatment recommendations and suggested that although 2 months regimen of rifampin plus pyrazinamide is effective and well tolerated amongst general population as compared to isoniazid alone, it should be discontinued due to high risk of hepatitis [100]. Hence, newer chemotherapeutic agents are needed urgently to target LTBI. So in the long run, effective immunotherapy along with chemotherapy remains major breakthrough in the management of LTBI.

CONCLUSION AND FUTURE RESEARCH: LTBI represents one of the major challenges in gaining control over TB worldwide. The state of bacillus and bacillary response in latency is still unknown which poses a great challenge for developing a prospective animal model that truly mimics human LTBI. *In vitro-in vivo* correlation has helped to our understanding of improved models of LTBI. Although recent advances in molecular biology tools like RT-PCR, DNA microarray has led to identification of various gene products, the lack of well defined gene targets against LTBI remains another obstacle for development of new diagnostic and chemotherapeutic agents. Elimination of LTBI and prevention of relapse remains important challenging aspects of chemotherapy.

The identification of new biomarkers would revolutionize development of anti-TB agents. The future of clinical LTBI management will use biomarkers to improve patient care through better diagnosis and hopefully to achieve greater success in treatment.

Developing novel anti-TB vaccines could complement or replace existing BCG vaccine which itself constitutes a global research area to fight TB. It is a clear and more realistic scenario that one has to think of combination of vaccines to prevent infection in unexposed individuals and chemotherapy to cure disease in infected population for complete eradication of TB from human beings. Improved therapies for TB must not only be discovered and developed, but must also be made affordable, accessible and adopted throughout the world to help eliminate TB as a major public health problem. Future studies are needed to explore following questions and to evaluate potential activity of anti-TB drugs and vaccines.

Some unsolved outstanding questions for future research:

1. Where do latent bacilli are located?
2. What is metabolic and replicative state of pathogen, and how long does they persist?
3. How latent bacilli reactivate?
4. Is it possible to develop and validate animal models and biomarkers for LTBI?
5. What are the possible strategies to develop potential anti-TB drugs and shorten LTBI therapy?
6. How could we develop more safe and efficacious drug regimen for TB-HIV co-infection?

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