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Review:

Global Threats and the Control of Multidrug-Resistant Tuberculosis

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About one-third of the world's population has been infected with *Mycobacterium tuberculosis*. Active disease develops in about 9 million people per year, and tuberculosis is responsible for 2 million deaths per year. The disease caused by this *bacterium*, tuberculosis (TB), remains one of the leading causes of mortality caused by infection worldwide and is a major threat to global health. The situation of TB is recently exacerbated by the emergence of highly drug-resistant forms of the disease-causing pathogen and synergy with human immunodeficiency virus/acquired immune deficiency syndrome, which greatly increases the risk of latent *M. tuberculosis* infection progressing to active disease. Multidrug-resistant (MDR) tuberculosis is defined as disease caused by strains of *M. tuberculosis* that are at least resistant to isoniazid and rifampicin; extensively drug-resistant (XDR) tuberculosis refers to disease caused by MDR strains that are also resistant to any fluoroquinolone and any of the injectable drugs used in treatment with second-line anti-tuberculosis drugs (amikacin, capreomycin, and kanamycin). MDR- and XDR-TB are serious threats to the progress that has been made in the control of tuberculosis worldwide over the past decade. In this review, we focus on threats of MDR-TB and the research and development of improved diagnostics, new chemotherapeutic agents, and vaccine candidates for MDR-TB.

Keywords: surveillance, diagnostics, control strategy, chemotherapeutic intervention, vaccine research and development

1. Introduction

Tuberculosis (TB), one of the oldest diseases known to affect humans, is a major cause of death worldwide. This disease, which is caused by acid-fast bacteria of the *Mycobacterium tuberculosis* complex, usually affects the lungs, although other organs are involved in up to one-third of cases. Characteristic features include man-to-man airborne transmission, a prolonged subclinical latency period between the initial infection and overt disease, a granulomatous response that is a compact organized collection of macrophages, associated with intense tissue inflammation and damage. If properly treated, TB caused by drug susceptible strains is curable in virtually all cases. If untreated, the disease may be fatal within 5 years in 50-65% of cases. Transmission usually takes place through the airborne spread of droplet nuclei produced by patients with infectious pulmonary TB [1].

About 15 million (>25%) of 57 million annual deaths worldwide are estimated to be related directly to infectious diseases. Among them, TB is still a major threat to global health, recently exacerbated by the emergence of drug-resistant strains of *M. tuberculosis* and coinfection with human immunodeficiency virus (HIV)/acquired immune deficiency syndrome (AIDS). The global plan to stop "TB: 2006-2015," which set out a vision of halving the prevalence of and mortality caused by the disease by 2015, followed by eliminating the disease as a public health problem by 2050 declared by the Stop TB Partnership in 2006 [2]. This vision depends on the development of improved diagnostics, better treatment by using novel anti-TB agents, and more effective vaccination. Recently, active translational research pipelines directed toward these goals have progressed, but improved understanding of the fundamental biology of this complex disease, including the causative pathogen and host responses, will promise to be the key to radical advances in TB control [1].

TB is one of the most devastating re-emerging diseases. The re-emergence of TB was fueled by the immune deficiencies of people with HIV/AIDS, which greatly increases the risk of latent *M. tuberculosis* infection progressing to active disease, and being transmitted to other. Inadequate courses of anti-TB chemotherapy compound the problem, leading to the emergence and spread of drug-resistant and multidrug-resistant (MDR) strains of *M. tuberculosis*, and a need for more expensive treatment strategies such as directly observed therapy. It has been known for over a century that TB is a disease of poverty, associated with crowding and inadequate hygiene. The continuing expansion of global populations living in poverty makes TB more difficult to control [3].

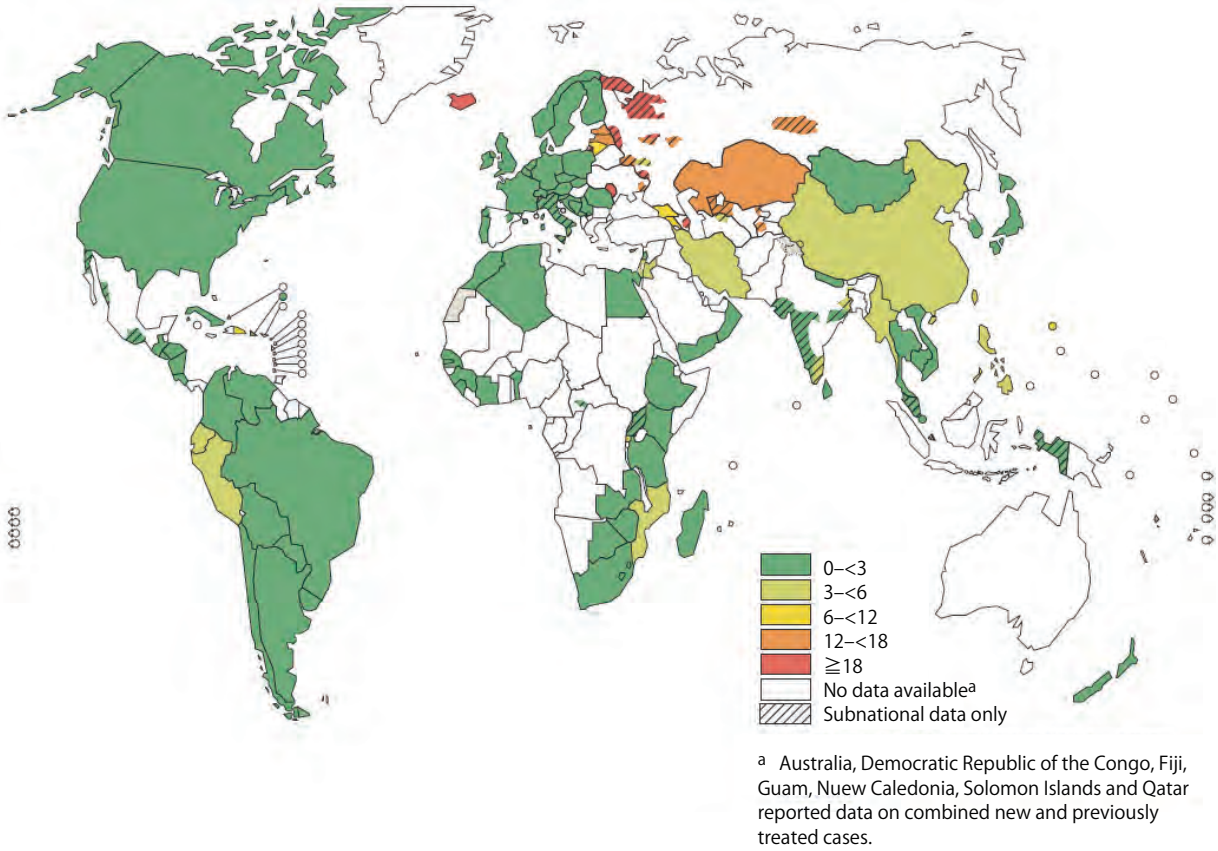

Fig. 1. Proportion of MDR-TB among new TB cases, 1994-2009. The proportion of MDR-TB among new TB cases reported ranges from 0% to 28%. The following 27 countries are responsible for 85% of the world's estimated cases of MDR-TB and are classified as countries with a high burden of MDR-TB: China, India, Russia, Pakistan, Bangladesh, South Africa, Ukraine, Indonesia, Philippines, Nigeria, Uzbekistan, Democratic Republic of Congo, Kazakhstan, Vietnam, Ethiopia, Myanmar, Tajikistan, Azerbaijan, Moldova, Kyrgyzstan, Belarus, Georgia, Bulgaria, Lithuania, Armenia, Latvia, and Estonia [9].

2. Definition of Multidrug- and Extensively Drug-Resistant Tuberculosis

Multidrug-resistant (MDR)-TB is defined as disease caused by strains of *M. tuberculosis* that are at least resistant to both isoniazid (INH) and rifampicin (RIF). Extensively drug-resistant (XDR)-TB refers to disease caused by MDR strains that are also resistant to any fluoroquinolone and any of the injectable drugs used in treatment with second-line anti-TB drugs, such as amikacin, capreomycin, and kanamycin. MDR- and XDR-TB are serious threats to the progress that has been made in the control of TB worldwide over the past decade [4].

3. Epidemiology

About 2 billion people in the world today are latently infected with *M. tuberculosis*. There were 9.4 million new TB cases (139 per 100,000 population) and 1.7 million deaths from TB in 2009 [5]. It has been reported 24 thousand (19 per 100,000 population) of new TB cases and 2.2 thousand deaths by TB in Japan, 2009 [6]. The public health surveillance system for TB in Japan is based on the law regarding infectious disease prevention and medical care for the patients. TB is legally classified as a type 2 disease, and therefore the clinical doctor responsible for the patient must report the information to the regional public health center immediately. The data are collected by the prefecture and then finally by the national center, the Tuberculosis and Infectious Diseases Control Division, Health Service Bureau, Ministry of Health, Labour and Welfare of Japan. National Tuberculosis Treatment Research Unit and laboratory centers of Japan reported and analyzed the results of drug susceptibility testing of *M. tuberculosis* [6, 7].

The proportion of MDR-TB among new TB cases reported ranges from 0% to 28% (**Fig. 1**). In 2009, the annual number of new patients with MDR-TB was estimated to be 0.5 million (320 in Japan, 2005) and XDR-TB was an estimated 50 thousand cases globally (100 in Japan, 2005) [8, 9]. The following 27 countries are responsible for 85% of the world's estimated cases of MDR-TB and are classified as countries with a high burden of MDR-TB: China, India, Russia, Pakistan, Bangladesh, South Africa, Ukraine, Indonesia, Philippines, Nigeria, Uzbekistan, Democratic Republic of Congo, Kazakhstan, Vietnam, Ethiopia, Myanmar, Tajikistan, Azerbaijan, Moldova, Kyrgyzstan, Belarus, Georgia, Bulgaria, Lithuania, Armenia, Latvia, and Estonia [9, 10].

Table 1. Mechanisms of drug action and resistance of mycobacteria.

Drug	MIC (μg/mL)	Genes associated with resistance	Gene function	Role	Mechanisms of action	Mutation frequency (%)
Isoniazid (INH)	0.02-0.2	*kat G* *inhA* *ndh* *ahpC*	Catalase peroxidase Enoyl ACP reductase NADH dehydrogenase Alkyl hydroperxidase	Prodrug conversion Drug target Modulator of INH activity Marker of resistance	Inhibition of mycolic acid synthesis Other multiple effects on lipids, carbohydrates, NAD metabolism, and cell wall assembly	20-80 15-40 10 10-15
Rifampicin (RIF)	0.5-2	*rpoB*	RNA polymerase	Drug target	Inhibition of transcription	95
Pyrazinamide (PZA)	16-50	*pncA*	Nicotinamidase Pyrazinamidase	Prodrug conversion	Acidification of cytoplasm and inhibition of membrane energy	70-95
Ethambutol (EMB)	1-5	*embCAB*	?	Drug target	Inhibition of arabinogalactan synthesis	50-65
Streptomycin (SM)	2-8	*rpsL* *rrs*	12S ribosomal protein	Drug target	Inhibition of protein synthesis	50-60

MIC: minimum inhibitory concentration
ACP: acyl carrier protein
NADH: reduced form of nicotinamide adenine dinucleotide
NAD: nicotinamide adenine dinucleotide
FAS: fatty acid synthase

MDR-TB can be treated and cured. However, treatment regimens are complicated, lengthy, and expensive. Medications that are currently available can produce crippling side effects and are less effective than drugs for non-resistant TB. If left untreated, however, MDR-TB not only kills the patients but can spread to other people, where it may develop additional drug resistance. Strains of XDR-TB that are resistant to three or more of the second-line drugs used to treat MDR-TB have reached epidemic proportions in several areas. One outbreak of XDR-TB in KwaZulu-Natal, South Africa, killed 74 of 78 patients within a matter of weeks, sparking fears that XDR-TB could spread rapidly and lethally, particularly in areas with high prevalence of HIV infection. In September 2006, the World Health Organization issued an alert regarding the emergence of XDR-TB [9].

National TB programs are challenged by MDR-TB. Globally, fewer than 2% of estimated cases of MDR-TB are reported to the World Health Organization (WHO) and managed according to international guidelines [11]. The vast majority of remaining cases are probably never properly diagnosed or treated, further propagating the epidemic of MDR-TB. The situation is further worsened by the epidemic of HIV, especially in Africa [10].

4. Mechanisms of Drug Resistance in Tubercle Bacilli

Drug resistance arises due to the improper use of chemotherapeutic agents of drug-susceptible TB patients. This improper use is a result of a number of actions, including administration of improper treatment regimens by health care workers and failure to ensure that patients complete the whole course of treatment. Essentially, drug-resistance arises in areas with poor TB control programs [4].

Resistance is a phenotype, the ability of pathogens to survive in the presence a drug at a concentration that normally kills and/or inhibits growth. Resistance is caused by mutation of the target gene ("genetic resistance") and can be distinct from tolerance ("phenotypic resistance") that is a conditional phenotype mediated by the physiological/metabolic state of the bacilli/pathogens. Thus, a phenotypic resistance is defined as drug resistance in stationary-phase, persistent, and dormant bacilli. Antimicrobials usually act against actively growing bacteria but not against non-growing forms. The lack of susceptibility of the non-growing bacteria to antimicrobials is due to changes in bacterial metabolism or physiological state and is therefore called as a phenotypic resistance. The phenotypic resistance plays an important role in subclinical/asymptomatic latent TB infection in which dormant bacilli are existed.

Drug resistance in *M. tuberculosis* can be either intrinsic or acquired. Intrinsic resistance refers to nonsusceptibility due to unique features of *M. tuberculosis*, such as its natural resistance to penicillin or clarithromycin. Acquired resistance refers to susceptible *M. tuberculosis* becoming resistant to drugs as a result of genetic mutations. The genetic basis of antimicrobial resistance in *M. tuberculosis* to date indicates that the MDR phenotype is the result of accumulative mutations of target genes and proteins rather than the acquisition of an MDR transfer factor. **Table 1** summarizes mechanisms of drug action and resistance of mycobacteria [12, 13].

Since the time when antimicrobial drugs were first introduced to treat infectious diseases, drug resistance has emerged. Thus, the development of resistance is an inevitable consequence of the clinical use of antimicrobial drugs. This is because the large number of bacterial cells in populations allows for the selection of mutants that are resistant to the drugs. Genetic resistance is due to a change in the genotype resulting in a drug-resistant phenotype of bacteria that can be passed on to subsequent generations. A better understanding the mutations participating in drug resistance not only clarifies the mechanism of drug resistance and action of drugs but also facilitates

Table 2. Technologies for detection of drug-resistant *Mycobacterium tuberculosis*.

Technology	Description	Product	Skill/Training
Liquid culture	Broth-based culture systems detect MTB and can be used for drug susceptibility testing	BacT/ALERT 3D, MGIT	Extensive
Microscopic observation drug susceptibility (MODS)	Manual liquid culture technique uses an inverted light microscope and microscopy skills to detect MTB be used for drug susceptibility testing	Non-commercial	Extensive
Molecular line probe assay	Strip test detects MTB and genetic mutations indicating IHN and/or RIF resistance	GeoType MTBDR, MTBDRPlus, INNO-LiPA Rif.TB	Moderate
Automated detection and screening	Automated sample processing: DNA amplification and detection of MTB and RIF resistance	Cepheid Gene Xpert	Minimal

MTB: *Mycobacterium tuberculosis*
MGIT: Mycobacteria Growth Indicator Tube

simple and rapid detection of drug-resistant *M. tuberculosis* by techniques of molecular biology [14].

5. Detection of Drug-Resistant *M. tuberculosis*

The methods to test the susceptibility of mycobacteria generally accepted are based on the growth of mycobacteria on solid or in liquid media containing a specified concentration of a single drug (**Table 2**) ("gold standard"). Classical cultivation methods in the addition of drug to detect inhibition of growth show advantages, including 1) less expensive, affordable in resource-limited setting, 2) the clinical relevance of results predicting treatment success/failure for decades, 3) detection of resistance regardless of the precise mechanism and/or molecular basis, and 4) unaffected by certain mutations that may not cause resistance [15].

The microscopic-observation drug-susceptibility (MODS) assay [15] is considered to be an inexpensive tool for the bacteriologic diagnosis of TB and the detection of drug resistance. This method is based on direct inoculation of the selective Middlebrook 7H9 liquid culture medium in 24-well plates with a sputum specimen. Detection of the typical cord formation ("microcolonies") of *M. tuberculosis* in the wells on microscopic examination (under an inverted light microscope) constitutes the basis of diagnosis. Growth (or the lack) in drug-containing wells, as compared with growth in drug-free wells, is the basis for reporting the results as "susceptible" or "resistant" to anti-TB agent. The reported sensitivity of this method in the recovery of mycobacteria from sputum specimens was higher than the sensitivity of either the MB/BacT automated mycobacterial system or traditional culture. The major difficulty in the implementation of the MODS assay or any other new cultivation method is that of biosafety and requirement for extensive training/skill.

Recently, molecular biology-based detection of genetic mutations linked to drug resistance has been developed, and it has several advantages over the conventional phenotypic culture method, including molecular line probe assay and automated detection of *M. tuberculosis* and screening for drug-resistant *M. tuberculosis* [14, 16, 17]. Advantages include 1) speed (results may be available within hours or days vs weeks and/or months by cultivation), 2) better reproducibility, especially in low-level resistance, and 3) ability to work with poorly growing cultures.

It has been reported that a new automated nucleic acid-amplification test that may allow a relatively unskilled health care worker to diagnose TB and detect resistance to a key anti-TB drug, rifampin (RIF), within 90 minutes. A large, well-conducted, multicountry study evaluated an automated tuberculosis assay (Xpert MTB/RIF) for the presence of *M. tuberculosis* and resistance to RIF. With a single test, this assay identified 98% of patients with smear-positive and culture-positive TB (including more than 70% of patients with smear-negative and culture-positive disease) and correctly identified 98% of bacteria that were resistant to RIF. This test and others that are likely to follow have the potential to revolutionize the diagnosis of TB and detection of RIF resistance [17]. The assay has several critical advantages over conventional nucleic acid-amplification tests. The Xpert MTB/RIF assay is simple to perform with minimal training, is not prone to cross-contamination, requires minimal biosafety facilities, and has a high sensitivity in smear-negative TB. However promising these findings, issues involving the MTB/RIF assay may limit its utility. These issues include its high cost and limitations in testing only for resistance to RIF but not INH, PZA, and EMB of *M. tuberculosis*, a platform that detects a relatively small number of mutations.

6. Chemotherapeutic Intervention for TB and Development of New Anti-TB Drugs

Treatment of TB due to drug-susceptible disease requires at least 6 months of a cocktail therapy by standard first-line anti-TB agents, including isoniazid (INH), rifampicin (RIF), ethambutol (EMB) and pyrazinamide (PZA), based on a strategy of directly observed treatment, short course (DOTS). To control TB, the World Health Organization (WHO) and the International Union against Tuberculosis and Lung Disease (IUATLD) recommend the DOTS strategy, which has five elements: political

Table 3. Goals of anti-tuberculosis therapy.

1. Safety and simplify the treatment of active, drug-susceptible TB
2. Improve efficacy and safety and shorten duration of therapy for drug-resistant TB
3. Develop drugs for TB coinfected with HIV to avoid the induction by RIF of CYP450 that metabolizes anti-HIV drugs
4. Shorten therapy of latent TB infection

TB: tuberculosis
HIV: human immunodeficiency virus

Table 4. Anti-tuberculosis drug candidates in clinical development.

Drug	Developers	Mechanisms	Stage	Active against MDR-TB
Diamine SQ-109	Sequella	Inhibits cell wall biosynthesis	Phase 1	Yes
Diarylquinoline TMC207	Johnson & Johnson	ATP synthase inhibitor	Phase 3	Yes
Nitroimidazoles		Inhibit cell wall lipid synthesis		
OPC-67683	Otsuka		Phase 2	Yes
PA-824	Global TB Alliance		Phase 2	Yes
Fluoroquinolones		Inhibit DNA replication and transcription		
Gatifloxacin	Bayer, Global TB Alliance, CDC		Phase 3	Yes
Moxifloxacin	WHO-TDR, Lupin		Phase 3	Yes

MDR-TB: multidrug-resistant tuberculosis
ATP: adenosine triphosphate
CDC: Centers for Disease Control and Prevention
WHO: World Health Organization
TDR: Tropical Disease Research
DNA: deoxyribonucleic acid

commitment, diagnosis primarily by sputum-smear microscopy among patients attending health facilities, short-course treatment with effective case management (i.e., direct observation), regular drug supply, and systematic monitoring to assess outcomes of every patient started on treatment. Standard short-course regimens, which involve an initial phase of INH, RIF, PZA and EMB for the first 2 months followed by a continuation phase of INH and RIF for the last 4 months, can cure more than 95% of cases of new, drug-susceptible TB. Between 1995 and 2008, a total of 36 million people were treated successfully with the use of the DOTS approach, and 6 million lives were saved [4]. By contrast, treatment of MDR-TB can require more than 18 months of therapy. Identification of drugs that shorten the duration of treatment and thereby improve adherence is key to improving active TB treatment, decreasing demands on national TB control programs, and preventing further emergence of drug-resistant bacilli [1, 4]. Goals of TB chemotherapy are shown (**Table 3**).

MDR-TB is a form of TB that is difficult and expensive to treat and fails to respond to standard first line drugs. While drug-resistant TB is generally treatable, it requires extensive chemotherapy (up to two years of treatment) with second-line anti-TB drugs, which are more costly than first-line drugs and may produce adverse drug events that are more severe. Drug-resistant TB has required the most effective use of existing second-line anti-TB drugs, such as aminoglycosides, polypeptides, fluoroquinolones, thioamides, cycloserine/terizidone, and para-aminosalicylic acid, and other antimicrobials available to treat drug-resistant TB and develop new chemotherapeutic agents, particularly against MDR/XDR-TB. These situations demand research to improve therapeutic outcomes and optimize current treatments. Fortunately, over the past five years, TB drug development has shown remarkable progress [18–20]. For the first time in many years, there is now a coordinated portfolio of promising new compounds at every level of drug development, and several new drugs for TB therapy are being assessed in clinical trials (**Table 4**). Most of them can be effective against drug-resistant strains and shorten the duration of treatment.

Diamines:

The most promising diamine candidate is SQ-109 [21], EMB analogues with 1,2-diamine pharmacophere. SQ-109 inhibits the cell wall synthesis of drug-susceptible and -resistant mycobacteria. Because resistance rates of SQ-109 are low, it is considered that two gene changes are required for the resistance, and SQ-109 may have more than one target in *M. tuberculosis*. No serious adverse effects were reported in a phase 1 study.

Diarylquinolines:

The most active diarylquinolone is TMC207 [22], which is being assessed in phase 3 clinical trials. TMC207 inhibits adenosine triphosphate (ATP) synthase of mycobacteria and is effective against both drug-susceptible and -resistant *M. tuberculosis*. No cross-resistance to current anti-TB agents is thought, because the drug target differs from the available agents. No serious adverse events were reported.

Nitroimidazoles:

Nitroimidazoles have derived from the bicyclic nitroimidazofurans that were originally developed as

Table 5. Vaccine candidates for tuberculosis.

Candidate	Developers	Composition	Mode of action
Viral vectors			
MVA85A	University of Oxford	Ag85, mycolyl transferase, expressed by vaccinia virus vector, modified vaccinia Ankara	Boost response to BCG
Ad35 TB-S	Aeras, Crucell	Ag85 and Tb10.4, a subfamily of ESAT-6, expressed by adenovirus vector	Boost response to BCG
Protein			
Mtb72F	GlaxoSmithKline, Corixa, Aeras	Recombinant fusion protein in adjuvant	Boost response to BCG
Hybrid-1	Statens Serum Institut	Ag85-ESAT-6 fusion protein in adjuvant	Boost response to BCG
HyVac4-IC31	Statens Serum Institut, Aeras, Intercell	Ag85-Tb10.4 fusion protein in adjuvant	Boost response to BCG
Modified BCG			
rBCG30	UCLA, Aeras	Recombinant BCG overexpressing Ag85	Augmented immunogenicity of BCG
rBCGUre:Chly-	Max Planck Institute	Recombinant BCG for enhanced MHC class I presentation, induction of cytotoxic T lymphocytes	Augmented immunogenicity of BCG
HyVac4-IC31	Statens Serum Institut, Aeras, Intercell	Ag85-Tb10.4 fusion protein in adjuvant	Boost response to BCG
Inactivated whole cells			
Mycobacterium vaccae	AnHui Longcom Biologic Pharmacy	Environmental nonpathogenic mycobacteria	Adjunct to chemotherapy, varying results
Mycobacterium w	Immuvac by Cadila Pharmaceuticals	Environmental nonpathogenic mycobacteria	Adjunct to chemotherapy

UCLA: University of California, Los Angeles
ESAT: early secreted antigen target
BCG: bacillus Calmette-Guérin
MHC: major histocompatibility complex

chemotherapeutic agents for cancers. These are effective for actively growing and dormant *M. tuberculosis* (latent TB infection) and includes OPC-67683 and PA-824, and for drug-susceptible and -resistant *M. tuberculosis*. These are now in phase 2 clinical trials.

OPC-67683 [23]; OPC-67683 inhibits biosynthesis of mycobacterial lipids, such as mycolic acid. The anti-mycobacterial activity of OPC-67683 in vitro was better than INH and PA-824. No cross-resistance and serious adverse events were observed.

PA-824 [24]; PA-824 inhibits the synthesis of proteins and cell wall lipids and also produces nitric oxide that is needed to control growth of *M. tuberculosis*. This agent is active against both drug-susceptible and -resistant organisms and limited to *M. tuberculosis* complex. No cross-resistance was observed. No serious adverse events were found, although mild toxicity to kidneys was seen.

Fluoroquinolones:

Fluoroquinolones [25] are known as second-line drugs for TB. Among them, moxifloxacin and gatifloxacin are candidates for shortening the duration of treatment, because they have low minimum inhibitory concentrations (MICs) and strongest bactericidal activity. Both moxifloxacin and gatifloxacin are currently in phase 3 trials. These inhibit bacterial DNA gyrase that is essential for DNA supercoiling and necessary for chromosomal replication. No cross-resistance to first-line agents was observed, and thus these were effective for MDR-TB. These show certain adverse events, such as nausea, vomiting, myalgia, tremor, insomnia, and dizziness, but not irreversible and life-threatening events. In addition, prolongation of QT intervals found by electrocardiogram may be associated with ventricular tachycardia and dysglycemias, including hyperglycemia and hypoglycemia. Fluoroquinolones are contraindicated for general use in patients with $<$18 years old and pregnant women.

7. Prevention of TB: Vaccine Research and Development

Prevention is better than cure. Ultimately, global eradication of TB will require a new effective vaccine. Although vaccination with bacillus Calmette-Guérin (BCG), a live attenuated *M. bovis*, has been shown to decrease the risk of severe forms of childhood TB, but its efficacy against pulmonary TB of adults is questionable, which remains the main source of transmission. One of the major obstacles to develop effective vaccines combating TB is that *M. tuberculosis* establishes intracellular infection. Vaccines that merely induce antibodies cannot prevent the disease. Thus, a critical part of TB vaccine development is directed toward the induction of cell-mediated immunity mediated by protective type 1 helper T (Th1) cell responses. New TB vaccines better than the current BCG vaccines are greatly needed to control the disease, which kills approximately 2 million persons in the world today.

There are two potential vaccination strategies against TB: 1) vaccines can be given before infection (pre-exposure, prophylactic) to prevent infection (or more probably, disease) or 2) post-exposure (therapeutic) to eliminate or contain latent TB and prevent reactivation. Therapeutic vaccines can also be used in combination with drug therapy. The development of a more effective,

better standardized, affordable vaccine with durable activity and fewer adverse effects is a major priority.

There are now more than 10 vaccine candidates in development, and they use different antigens and delivery strategies. Vaccines and strategies under development and in clinical trials include 1) improved/recombinant BCG, 2) attenuated auxotroph *M. tuberculosis*, 3) subunit/component vaccines, and 4) DNA vaccines. These are based on a prime by BCG-boost system, a two-stage immunization approach (prime-boost). Candidate vaccines [12, 26, 27] have demonstrated activity in animal models that is equal to or superior to that of BCG, and human trials are underway (**Table 5**).

Among them, a leading vaccine candidate, modified vaccinia Ankara (MVA85) expressing antigen 85A encoding an important enzyme of lipid metabolism, mycolyl transferase, has been studied in BCG-unvaccinated and -vaccinated subjects. MVA 85A in a clinical trial induced a robust Th1 cell response (interferon-γ and interleukin 2) lasting more than 50 weeks. Th1 responses can lead to protection against *M. tuberculosis* infection. MVA 85A was shown to be safe and highly immunogenic in phase 1 trial, leading the way to larger trails in endemic areas [28].

Mtb 72F represents a fusion protein comprising antigens Rv0125 and Rv1196 formulated in an oil-in-water emulsion with an adjuvant comprising lipopolysaccharide and a triterpene glycoside (AS02) produced by Glaxo-SmithKline [29]. It triggers a strong memory response in the immune system. When properly activated, memory cells confer long-lasting immunity. The fused protein and a new kind of adjuvant, AS02, have previously shown to be safe and effective in clinical trials.

However, there is no identified surrogate marker for protection; identification of an improved vaccine will require long-term efficacy trials in humans. Thus, we are facing the difficulty in the research and development of novel vaccines for TB. An effective vaccine would be the most powerful tool for preventing TB and drug resistance, but a vaccine is not anticipated anytime soon. We must invest in research and development for better tools while maintaining the efficacy of the tools we have available today.

8. Concluding Remarks

The current strategy for TB control is based on reducing the spread of infection through the effective treatment of individuals with active disease and the vaccination of children. The WHO has initiated the DOTS campaign in many regions; however, this program has not been able to control the global TB epidemic or prevent the incidence of MDR strains of *M. tuberculosis* [1]. To control MDR-TB, intensified research on new diagnostic tests, drugs, and vaccines are needed, as described in this review. Scientists and industries need to develop radically improved tools for TB elimination, including new diagnostics, drugs, and vaccines.

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Conflict of Interest

The authors have declared that no conflict of interest exists.

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