Respiratory Disease Series:
Diagnostic Tools and Disease Managements
Series Editors: Hiroyuki Nakamura · Kazutetsu Aoshiba

Takefumi Saito
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Pulmonary Tuberculosis and its Prevention

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Kazutetsu Aoshiba, Ibaraki Medical Center, Tokyo Medical University
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This book series cover a variety of topics in respiratory diseases, with each volume providing an overview of the current state of knowledge, recent discoveries and future prospects for each disease. In each chapter the editors pose critical questions, which are often unresolved clinical issues. These are then discussed by the authors, providing insights and suggestions as to which developments need to be addressed. The series offers new information, which will inspire innovative ideas to further develop respiratory medicine. This collection of monographs is aimed at benefiting patients across the globe suffering from respiratory disease.

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Takefumi Saito • Masahiro Narita
Charles L. Daley
Editors

Pulmonary Tuberculosis and Its Prevention

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Editors

Takefumi Saito, MD, PhD
Respiratory Medicine
NHO Ibarakihigashi National Hosp
Ibaraki, Japan

Charles L. Daley, MD
National Jewish Health
Denver, CO, USA

University of Colorado
School of Medicine
Aurora, CO, USA

Masahiro Narita, MD
Division of Pulmonary
Critical Care and Sleep Medicine
University of Washington
Seattle, WA, USA

TB Control Program
Public Health – Seattle & King County
Seattle, WA, USA

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Preface

TB remains a global health crisis, as an estimate of deaths from TB, the leading cause of deaths due to the infectious disease until the COVID-19 pandemic emerged, has increased to more than 1.5 million people in 2020. The COVID-19 pandemic has substantially affected the healthcare systems in many countries disrupting services and delaying diagnosis and treatment of other medical conditions, including TB. These disruptions in services have led to dramatic decreases in TB notification globally which are projected to result in an increase in deaths and likely more transmission of *Mycobacterium tuberculosis*. It is imperative for clinicians to "think TB" and take appropriate actions to diagnose and treat TB disease, especially pulmonary TB to stop its spread in the community. In addition, evaluation and treatment of latent TB infection (LTBI) is crucial as a preventative strategy to reduce the incidence of TB. This book offers practical information to clinicians who evaluate and treat patients with pulmonary TB and LTBI. It addresses epidemiology, pathogenesis, the role of radiology, advances in diagnostics and treatment of pulmonary TB. Furthermore, preventive strategies such as diagnosis and treatment of LTBI and TB vaccination are also covered. We are grateful to the authors for their invaluable contributions.

Ibaraki, Japan — Takefumi Saito
Seattle, WA, USA — Masahiro Narita
Denver, CO, USA — Charles L. Daley

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Part I
Epidemiology and Pathogenesis

Chapter 1
Epidemiology: Who Develops Pulmonary TB? How Does an Understanding of Global TB Epidemiology Help Clinicians Manage their Patients with Pulmonary TB?

Akihiro Ohkado and Seiya Kato

Abstract Epidemiology of TB provides important information for clinical diagnosis. The risk of TB infection and disease development depends on exposure to bacilli, i.e., contact with infectious TB patients, host factors such as comorbidities, and socio-economic conditions. TB was called as the "white plague" in Western countries and "nation's disease in Japan" because of its high mortality. Improvements in social conditions and the introduction of effective anti-TB drugs dramatically reduced the burden of the disease in such countries; however, the global annual incidence remains to be approximately ten million and the total number of deaths is 1.4 million mainly in developing countries. Major global challenges for TB control include HIV-associated TB, multidrug-resistant TB, migrant issues, and the elderly population. Since the 1990s, the World Health Organization (WHO) has strengthened TB control with the DOTS strategy, followed by the Stop TB Strategy and the End TB Strategy. The targets of the End TB Strategy are: (1) to reduce the incidence rate of TB by 90% by 2035, (2) to reduce the absolute number of TB deaths by 95% by 2035, and (3) to eliminate catastrophic costs faced by TB-affected families by 2020.

Keywords Epidemiology · Risk · Infection · Incidence · Strategy

A. Ohkado
Department of Epidemiology and Clinical Research, Research Institute of Tuberculosis, Tokyo, Japan

S. Kato (✉)
Research Institute of Tuberculosis, Tokyo, Japan
e-mail: kato@jata.or.jp

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1 Introduction

Epidemiology of TB is an essential component for national health departments to formulate a control policy as it helps to prioritize several health issues dealt using or focusing on the policy. An epidemiological analysis of TB is also important for clinicians to efficiently manage and treat TB patients, as the risk of developing the disease, along with its clinical course, is highly associated with social factors and individual conditions.

1.1 The Role of Epidemiology in Clinical Diagnosis and Management

Clinicians begin by obtaining the medical history of a patient to assess the probability of a disease. Epidemiological evaluation, including the probability of disease occurrence, helps clinicians to efficiently make a diagnosis. For example, in the context of globalization, clinicians can assess foreign-born patients whose country of origin has a high burden of TB. Knowledge of the country-specific risk of TB might provide a hint for its diagnosis. Social and individual risk factors are also associated with the clinical course of TB, because TB requires uninterrupted treatment with regular drug administration for 6 months or more.

Social determinants for risk of infection are as follows: poverty, unstable diet, poor living conditions, air pollution, working environment, housing, congregate setting, homelessness, prison, and so on. Individual risk factors for developing active TB include HIV/AIDS, diabetes mellitus, hemodialysis, tobacco smoking, malnutrition, immunosuppression, and mental illness [1]. The details of these factors are discussed in the following sections.

1.2 The Implication of Clinical Practice for Public Health

It is also important and useful for clinicians to understand public health strategies and policies, providing insights into effective clinical practice for TB patients, which ultimately contributes toward reducing the burden of TB in society by cutting chains of infection.

In this chapter, the implications of epidemiology for clinical practice are discussed.

2 The Disease Process of TB

TB is an airborne infectious disease caused by certain acid-fast bacilli species, i.e., *Mycobacterium tuberculosis* complex (MTB). It can affect any part of the human body, but mainly affects the lungs, and the transmission route of MTB is mainly via air.

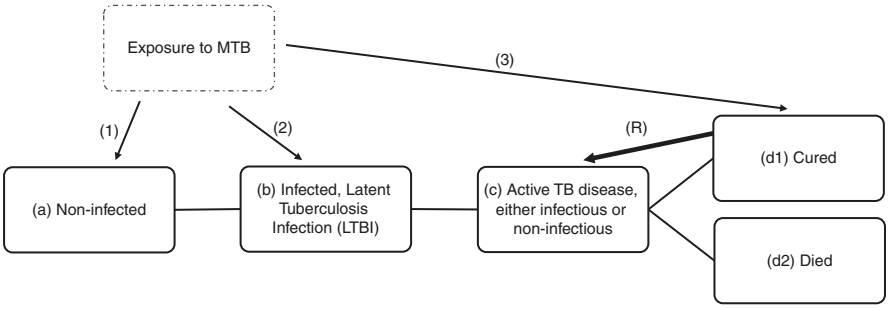


Fig. 1.1 A simplified tuberculosis epidemiological model in view of the pathogenetic pathway of tuberculosis [2, 3, 4]. *MTB* Mycobacterium tuberculosis*, TB tuberculosis*. (1) Exposure to MTB by the non-infected, (2) Exposure to MTB by the infected, (3) Exposure to MTB by the cured, (R) Relapse to active TB disease either by a recurrence or a reinfection. Courtesy of Dr. Hans Rieder

We describe a simplified pathogenic process of TB in Fig. 1.1. It begins from an individual being uninfected (a), progressing toward an infection, followed by a latent TB infection (LTBI) (b), next, the patient has clinically apparent TB (c), i.e., active TB disease which is either infectious or non-infectious, and finally, an individual being either cured or dead (d1, d2) [2, 3]. For the non-infected group (a), exposure to MTB may lead to LTBI (b) (1). In addition, exposure to MTB in LTBI patients (2) may lead to active TB disease (c) via reinfection. Cured TB patients (d1) can suffer from relapse (R) either by a recurrence of the initial MTB infection (i.e., by the same MTB strain) or by reinfection (i.e., by a different MTB strain) (3).

3 Exposure to MTB

One of the reasons why it is challenging to eliminate TB is its human-to-human airborne route of transmission. The pathogenesis of TB begins due to exposure to MTB, i.e., TB cannot be eliminated until the exposure to MTB is eliminated. Theoretically, the world could be TB-free if we could create and maintain an environment in which exposure to MTB is eliminated. However, the exposure to MTB has existed in the real world and is likely to continue.

According to Rieder, the definition of significant exposure to MTB is “contact between two individuals in sufficient proximity to allow conversation between them, or, within confined spaces, where the air exchange (ventilation) of the space has been incomplete between the visits of two persons” [3]. The human-to-human transmission of MTB may occur when, for instance, an active pulmonary TB patient coughs, shouts, speaks, sings, sneezes, or expels aerosolized droplets from their respiratory tract. The risk of exposure varies based on the index source case situation, including the frequency of cough, number of MTB in sputa, the environment (distance from the contact person, ventilation condition, space shared, duration of time of contact, etc.).

3.1 Risk Factors for Exposure to MTB

3.1.1 MTB Side Factors

MTB comprises multiple species, such as *M. tuberculosis*, *M. africanum*, *M. bovis*, and *M. bovis* BCG. The transmissibility of each species is reportedly different, for instance, *M. bovis* has lower human-to-human transmissibility than *M. tuberculosis,* however, the difference of transmissibility between *M. africanum* and *M. tuberculosis* remains unknown [2].

3.1.2 Prevalence of Infectious TB Cases in a Community

The risk of exposure to MTB in a community depends partly on the number or prevalence of infectious TB patients in a population (P) at a certain time. The prevalence of TB depends on two conditions, namely (1) the incidence of infectious TB patients in a defined period (I), and (2) the duration of infectiousness (D), i.e., $P = I \times D$. Assuming that the duration of infection is fixed under proper TB treatment, with an accurate notification system wherein minimal patients diagnosed with TB are missing, we can estimate the prevalence of TB in a population. If we miss diagnosing an infectious TB patient or delay the initiation of proper TB treatment, the patient will spread the infection in the community. In addition, inappropriate treatment of TB, i.e., either improper combination or inadequate doses of anti-TB drugs, or irregular drug intake by the TB patient will also cause an increase in the duration of infection [3].

3.1.3 Population Density and Environmental Conditions

It seems natural to observe the impact of living conditions, family size, and population density of the patients because the risk of exposure to MTB depends mainly on the proximity between the infectious TB patient, the susceptibility of the individual in contact, and the duration of the contact. It has been reported that residents or people who stay in congregate settings such as mines, nursing homes, homeless shelters, prisons, and hospitals had higher tuberculin skin test (TST) positive results. Contacts who are family members are likely to have the most intensive exposure to MTB when they share rooms and spend long periods of time with infectious TB patients [3].

Although MTB disperses quite instantly in outdoor conditions, indoor conditions do not allow them to disperse, therefore MTB remains in the air for a long time. Like other airborne transmission agents, MTB expelled by a TB patient in a limited space with improper ventilation may be able to retain its viability for quite a long time [3].

4 Infection with MTB

4.1 Definition, Measurement, and Mode of Infection

First, we need to clarify that it is difficult to clinically define the status of MTB infection, i.e., there is no clear demarcation between infection and non-infection with MTB ((a) and (b) in Fig. 1.1). MTB infection can be defined as "a case in which MTB bacilli invade into the host and grow, then pathologically formulate a primary complex, which can be immunologically detected by the multiplication of sensitized T-lymphocytes" [4]. It is impractical to identify a primary complex, i.e., a pathologic change in the primary infection site with the lymph node on which MTB grows during the first infection because the complex disappears spontaneously in many cases. Historically, quite a few clinicians have therefore tried to measure MTB infection from the immunological response of the body using the multiplication of sensitized T-lymphocyte responses.

TST was introduced by Robert Koch as a potential diagnostic tool for TB diagnosis in the late nineteenth century and has been used to measure MTB infection [3]. Since then, TST has been utilized globally as a diagnostic tool for MTB infection until recently. It is used to detect a delayed-type cell-mediated hypersensitivity immunological response to a purified protein derivative (PPD). The proteins in the PPD share the antigenicity of *M. bovis* BCG and some non-tuberculosis mycobacteria (NTM). Hence, the TST has relatively low specificity in populations with high BCG vaccination coverage and a high prevalence of NTM infection. In addition, the sensitivity of the TST is negatively affected by immunocompromised conditions such as HIV/AIDS [5]. Furthermore, performing the TST and reading the results vary based on the skills of those who perform the test; therefore, intra- and inter-reader variability remains a common issue.

Andersen *et al*. first reported a possible alternative method for diagnosing MTB infection using MTB-specific antigens, such as the culture filtrate protein 10 (CFP-10) and early secretory antigenic target 6 (ESAT-6) [6]. These antigens are specific to the MTB, as they are encoded by the region of difference 1 (RD1) genes that do not exist in *M. bovis* BCG or most of the NTM species, except *M. kansasii*, *M. marinum*, and *M. szulgai* [5].

The primary mode of MTB infection is the droplet-nuclei mode of infection, i.e., airborne infection. The droplet mode of infection is also possible to transmit MTB into the human respiratory tract system, however, it rarely occurs. MTB infection occurs only when the bacilli reach either the terminal or respiratory bronchioles, or the alveoli; unlike other microorganisms such as measles, the number of MTB in a droplet is very miniscule. The diameter of the droplet nuclei of MTB is estimated to be somewhere between 1 and 5 mcm; which allows particles to remain in the air for several hours [3].

4.2 The Risk of Infection with MTB

4.2.1 Estimated Risk of Infection with MTB

It is virtually impossible to measure the incidence of MTB infections in a population because it requires enrolling a large number of people to perform the TST as a tuberculin survey, which is impractical to do in the real world. Performing the TST and reading the results are skill demanding for examiners and labor-intensive for both examiners and examinees. Furthermore, it is challenging to conduct a sound tuberculin survey with high coverage of the BCG vaccination population owing to its cross-reactivity with *M. bovis* BCG. In response to these challenges for conducting repeated tuberculin surveys, the average annual risk of infection (ARI) has been calculated from the prevalence of infection at a given age, e.g., 10 years, in a population. The calculation of the average ARI from the prevalence of MTB infection is based on the average annual probability of escaping infection with MTB, not the average annual probability of infection with MTB [3]. The equation to calculate the average ARI with MTB between calendar time b and $b + a$ is as follows:

$$\mathrm{ARI} = 1 - \left(1 - \mathrm{P}_{b+a}\right)^{1/a}$$

where P_{b+a} is the prevalence of infection with MTB in a cohort at the time of conducting the survey, b is the calendar year the cohort was born, and a is the age of the cohort when the survey was conducted. Styblo *et al*. focused on the secular trends in the ARI of MTB to describe the epidemic course of MTB in several European countries [3]. They estimated that the average number of infections per infectious TB source was approximately 20 persons during the 2-year infectious period, and the ratio of incidence to the prevalence of TB patients was approximately 1–2 in the pre-chemotherapy era [7, 8]. According to these assumptions, when we observe 100 prevalent infectious TB patients in a population of 100,000 individuals, it indicates 1000 TB transmissions per year in the community, i.e., 1% of the population per year would be infected. The 1% ARI corresponds to approximately 50 incident infectious TB patients per year per 100,000 population, with an incidence to prevalence ratio of 1–2. This rule, the so-called "Styblo's rule," has been extensively used globally to estimate the annual incident infectious TB patient numbers. This rule itself came from the available data before the chemotherapy era; therefore, it does not go well with the current chemotherapy era because effective chemotherapy reduces the duration of infectiousness of TB patients. Cauthen *et al*. reported that in countries where intervention effectively cuts the chain of transmission, the number of transmissions caused by one case will be reduced. Thus, to produce a 1% ARI, a larger number of incident cases is required because the person-time of infectiousness is reduced [9].

4.2.2 MTB Side Factors

Although MTB is transmissible via air, its transmissibility is not as high as that of other viral airborne infectious diseases such as measles. Riley *et al*. reported that an average of 1–1.5 years of exposure to TB patients was required for a student nurse to become infected in the pre-chemotherapy era [10]. Snider *et al*. reported that anti-TB drug resistance did not seem to affect the risk of infection among contacts of TB cases if the source index patient was previously untreated, even if the patient expelled isoniazid- or streptomycin-resistant MTB [11].

4.2.3 Host Side Factors

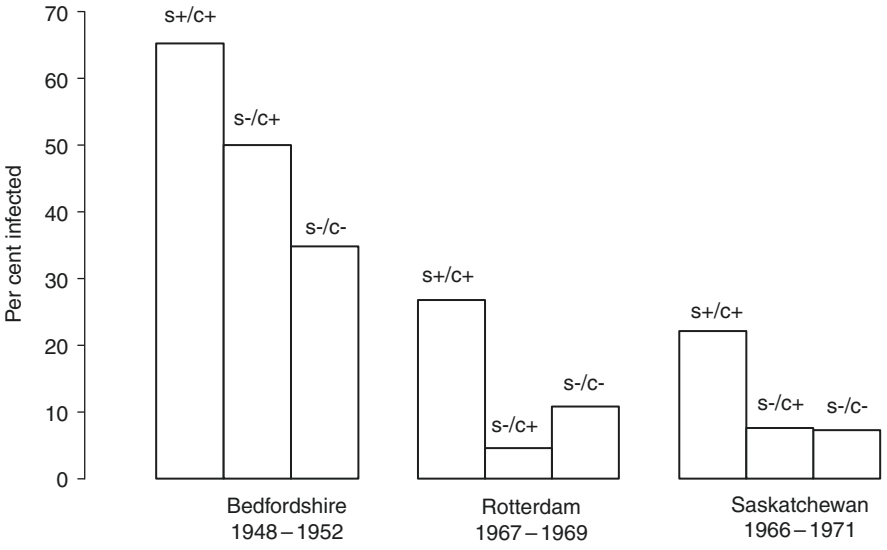
Factors Influencing the Risk of Infection Associated with the Infectious Source Case

Number of MTB in Sputum Specimens
Transmission of MTB, as a rule, occurs in patients with respiratory tract TB, and the risk of infection increases according to the number of MTB in sputum specimens [3]. For instance, the proportion of TST-positive individuals among household contacts of an active pulmonary TB case is much higher among those exposed to a sputum smear-positive pulmonary TB case than among those exposed to a sputum smear-negative pulmonary TB case. Hence, smear-positive pulmonary TB patients are the most important sources of MTB infection in the community (Fig. 1.2) [3].

It does not, however, exclude the possibility of infection from smear-negative pulmonary TB patients. Tostmann *et al*. stated that patients with smear-negative, culture-positive TB are responsible for 13% of TB transmission in the Netherlands [12].

The Severity of Cough in the Infectious Source Case
Cough increases the risk of infection, as many droplets are expelled from the respiratory tract via coughing. Cough etiquettes, such as covering the mouth and nose during coughing, effectively reduce droplets and droplet nuclei from infectious pulmonary TB patients.

Anti-TB Treatment in TB Patients
The early diagnosis and seamless initiation of chemotherapy have been the cornerstones of TB control around the globe. These are the primary countermeasures for reducing the risk of infection in a population. Chemotherapy drastically reduces the risk of infection in bacillary-positive TB patients within a few weeks [13]. The highest risk period of MTB infection for the contacts is right before the start of chemotherapy; hence, a delay in the diagnosis and treatment increases the risk of infection.



Shaw JB, et al. Am Rev Respir Dis 1954;69:724– 32
van Geuns HA, et al. Bull Int Union Tuberc 1975;50:107– 21
Grzybowski S, et al. Bull Int Union Tuberc 1975;50:90– 106

Fig. 1.2 Tuberculous infection among close contacts (Children <15 years) by Bacteriologic Status of Index case [3]. Courtesy of Dr. Hans Rieder

Factors Affecting the Risk of Infection Associated with an Exposed Host

The risk of MTB infection varies according to the situation of an exposed host. The order of risk of contracting MTB is probably the immunocompromised individuals, such as those with HIV infection, premature babies or infants, uninfected individuals, those infected with NTM bacilli, those with BCG vaccinated, and finally, those with previous TB infection [4].

Age and Sex-Specific Prevalence of TB Infection

The age-specific prevalence of TB infection based on the TST surveys conducted both in industrialized countries, where individuals suffered a high prevalence of TB infection, and in countries with a current high TB burden was similarly increased in children of both sexes, but was more rapid in adult males than in females [3].

It is well known that the age-specific prevalence of TB infection between the economically poor and economically prosperous individuals differs significantly; of course, the former group has a higher prevalence [3].

Contact with TB Patients and Environmental Conditions

The risk of MTB infection is higher in cases of close intimate contacts than in casual contacts; the closer and the longer the contacts stay with the source TB patients, the higher the risk of TB infection [14]. The density of MTB in the given space affects

the risk of infection in those who inhale the droplet nuclei with MTB; hence, it is essential to maintain considerable distance in a highly ventilated environment to prevent MTB infection. The best way is to open the window and allow natural ventilation. It should be emphasized that it is highly dangerous for medical staff to perform droplet-producing procedures such as bronchoscopy in a closed space without windows and under insufficient ventilation [15].

5 TB Disease

5.1 Definition of Active TB Disease

It is also challenging to clearly distinguish between the status of infection with MTB, i.e., the status of latent TB infection (LTBI) and that of active TB disease ((c) in Fig. 1.1). The decisions of clinicians and medical staff in clinical practice usually depend on the clinical manifestations; bacteriological test results such as a sputum-smear examination, a nucleic acid amplification test (NAAT), a culture test; and other clinical findings such as radiological and pathological findings. We are much less confident to treat patients unless we have positive bacteriological test results.

5.2 Progression to TB Disease

5.2.1 Natural History of Active TB Disease

The National Tuberculosis Institute, Bangalore, India, in 1974, reported that the outcomes of culture-positive pulmonary TB patients during the 5-year observation period was follows: 49.2% (62/126) had died, 32.5% (41/126) were converted to negative, 18.3% (23/126) remained sputum-positive, meaning that they were still alive but were expelling MTB in sputa [16]. Aoki estimated the progression from infection to active TB disease according to the disease duration, as shown in Table 1.1 [17, 18]. Tuberculous pleuritis and meningitis tend to manifest within a relatively short period after infection. In contrast, pulmonary TB tends to manifest a little later; approximately 80% manifest within 2 years after infection. Chiba conducted a prospective cohort study comprising 30,069 person-year observations for 30 years from 1939. He reported that the incidence rate of pulmonary TB among 1,192 BCG-unvaccinated natural TST converters was as high as approximately 16% within 1 year, dropped to approximately 1.1% per annum in 2–5 years, 1.0% per annum in 6–15 years, 0.3% per annum in 16–20 years, 0.2% per annum in 21–25 years, and 0.1% per annum in 26–30 years [19].

Table 1.1 Timetable of Tuberculosis

	Duration of time from infection to manifestation of tuberculosis	Number of cases	%
Positive conversion of tuberculin skin test			
	~3 weeks	3	5.4
	~7 weeks	51	91.1
	8 weeks ~	2	3.6
	(sub-total)	56	100
Tuberculous pleuritis			
	~2 months	7	5.1
	~7 months	94	69.1
	~12 months	23	16.9
	12 months ~	12	8.8
	(sub-total)	136	100
Tuberculous meningitis			
	~4 months	60	87.0
	~8 months	9	13.0
	(sub-total)	69	100
Bone and joint tuberculosis			
	~1 year	65	61.3
	~2 years	25	23.6
	2 ~ 6 years	16	15.1
	(sub-total)	106	100
Pulmonary tuberculosis			
	~1 year	85	60.7
	~2 years	25	17.9
	~6 years	27	19.3
	7 years ~	3	2.1
	(sub-total)	140	100

Source: References 17, 18

5.2.2 The Components Contributing to TB Disease

Rieder mentioned that three components contribute to TB disease: (1) progression from subclinical infection to disease within 5 years after the acquisition of infection; (2) TB disease from exogenous reinfection with MTB, i.e., progression from a second infection; and (3) TB disease from endogenous reactivation of a first infection after more than 5 years following the first infection [3]. Sutherland *et al.* described the estimated percentage contribution of active TB disease from these three components using data from 1951 to 1970 in the Netherlands, as shown in Fig. 1.3 [3]. The graph shows that TB due to a primary infection was the most significant contributor in the younger age group, aged 15–19 years, while it was a minor contributor to TB disease in the older age group, aged 60–64 years. The relative contributions to TB disease switched from exogenous reinfection to

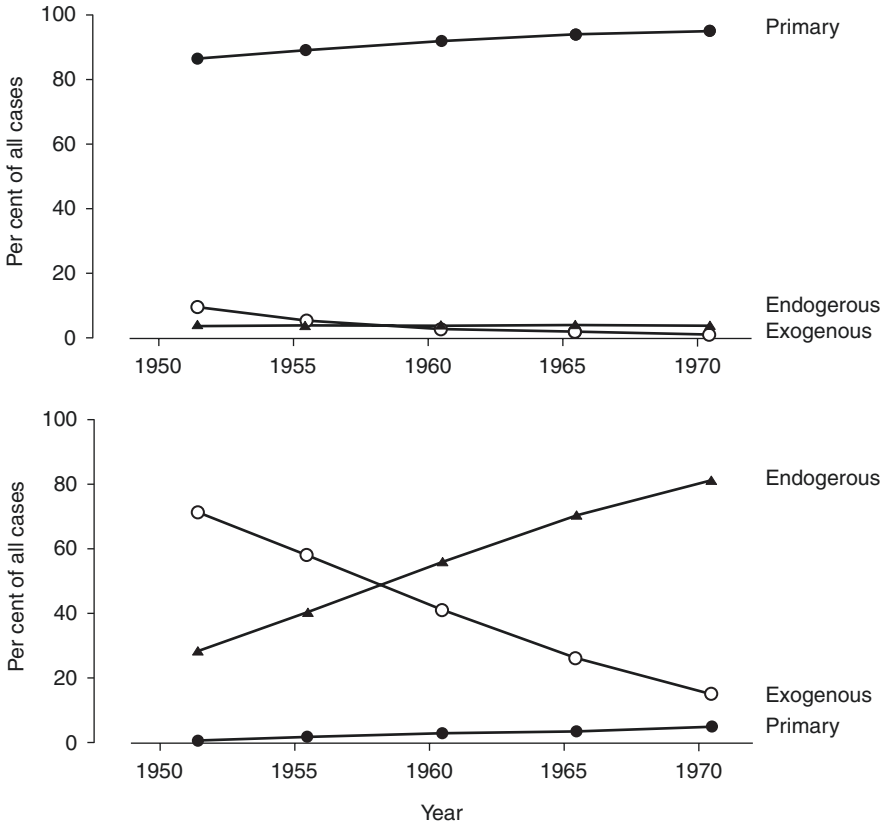


Fig. 1.3 Proportion of TB cases attribute to primary infection, exogenous reinfection, or endogenous reactivation, among males, by age, Netherlands [3]. Courtesy of Dr. Hans Rieder

endogenous reactivation in the older age group. This finding indicates significant implications of TB control interventions. Effective interventions should be implemented to reduce the duration of infectiousness of the index source if the percentage of active TB disease patients is high due to primary infection or exogenous reinfection within a community. In comparison, early diagnosis and complete treatment should be prioritized if the proportion of active TB disease is high due to endogenous reactivation.

5.2.3 Risk Factors for TB Disease after Infection

The pathogenicity of MTB is not strong enough to lead all infected patients toward active TB disease. At the same time, it is not weak enough to be eliminated by the immunological response of the human body, i.e., the cellular immunity of humans

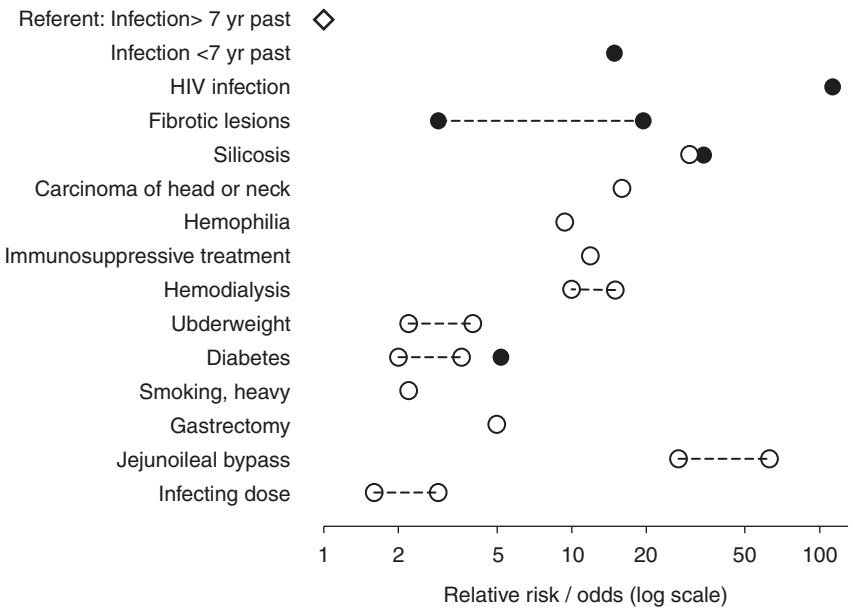


Fig. 1.4 Selected risk factors for TB given infection. The diamond represents the referent (infection that has occurred >7 years in the past), full circles are relative risks (from population-based studies), open circles are odds ratios (from case control studies), and dotted lines connecting circles indicate ranges in different studies [3]. Courtesy of Dr. Hans Rieder

is not strong enough to eradicate the MTB that invades the body. This allows MTB to survive in the human body until a deterioration occurs in the balance between the growth of the bacilli and the immunological response.

We need to consider the strength of the association between potential risks and progression to TB disease and the prevalence of such risks in a targeted population [3]. For instance, HIV infection is one of the most potent risk factors for active TB disease. Therefore, strengthening the TB control program combined with the HIV control program in a community with a high prevalence of TB and HIV infections should be prioritized. On the other hand, silicosis showed a very high risk of active TB disease, however, the prevalence of silicosis is usually low in the general population. Rieder summarized significant risk factors for TB disease, given that infection has already occurred, as shown in Fig. 1.4 [3].

MTB Side Factors

Number of MTB Bacilli Expelled

Interestingly, the higher the number of MTB bacilli expelled from an infectious source, the higher the probability of infection with MTB and the higher the risk of active TB disease. Grzybowski *et al*. showed that the risk of getting TB disease was

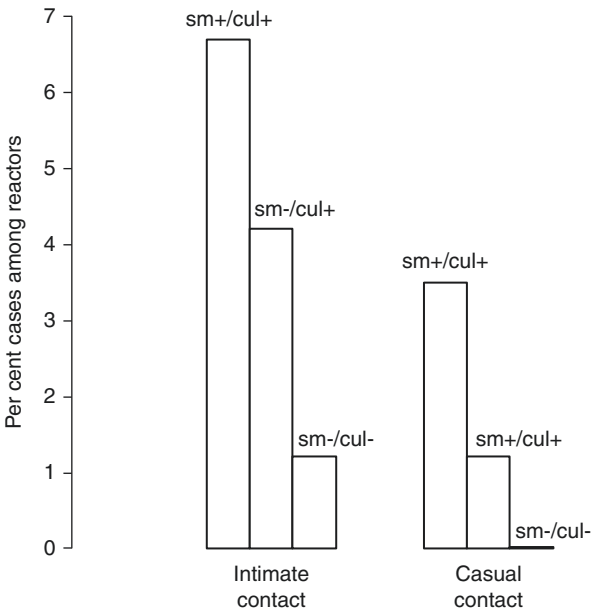


Fig. 1.5 Percentage of secondary cases among tuberculin-positive contacts, by Type of Source, Canada, 1966–1971 [3]. Courtesy of Dr. Hans Rieder

approximately twice as high among those from smear-positive TB patients than those from smear-negative culture-positive TB patients both in the intimate and in the casual contact groups (Fig. 1.5) [3, 20].

The Virulence of MTB

The risk of TB disease after infection varies according to MTB strains and their lineages. For instance, the risk of TB disease after *M. bovis* infection is much lower than that of TB disease after MTB infection [3]. de Jong *et al*. in the Gambia observed that Beijing lineage strains attributed to a significantly higher rate of TB disease progression with the hazard ratio of 16 (95% Confidence Intervals 2.8–89) soon after exposure to household contacts [21].

Host Side Factors

A number of risk factors for progression to active TB disease in the host after MTB infection have been identified, as indicated in Fig. 1.4 [3].

Contact with an Active TB Patient

Kamat *et al*. conducted a controlled study in South India and reported that there was virtually no difference in the relapse rates and attack rates of TB in the home care and sanatorium care groups [22]. The findings indicated that home care along with chemotherapy for TB is the choice of care and admission to a healthcare unit is not required for efficient TB care.

TST Results and BCG Vaccination

Grzybowski *et al*. reported very impressive findings on the average annual risk of developing active TB among Eskimos severely affected by TB. The average annual risk of developing active TB was 2,780 per 100,000 population among TST-positive reactors under 20 years of age without BCG vaccination compared with 1,720 per 100,000 population among TST-negative reactors; and with BCG vaccination, the annual risk of developing active TB dropped to 1,190 per 100,000 population [23].

Immunocompromised Conditions

HIV Infection: The HIV epidemic has drastically changed the global situation of TB. HIV infection negatively affects TB epidemiology, mainly through the following three areas. First, HIV infection enhances endogenous reactivation of MTB in patients with preexisting MTB infection. Second, it drives the progression from MTB infection to active TB in patients with preexisting HIV infection. Third, it increases the opportunities for MTB transmission to the general population from active TB patients who developed TB due to HIV infection [24]. Selwyn *et al*. reported that HIV infection enhanced the progression of TB disease among young intravenous drug users by stating that "7 of 49 HIV-positive patients progressed to TB during an average of 22 months of follow-up, yielding a rate of approximately 8 per 100 person-years. None of the 26 HIV-negative converters developed active tuberculosis during that period" [25]. Rieder highlighted that "HIV infection is the most powerful factor yet recognized in the progression to disease from pre-existing infection with *M. tuberculosis*. The relative risk of tuberculosis among HIV-seropositive women was 26 compared to seronegative women" [3]. The approximate life-long risk of active TB disease from LTBI patients is the annual risk among HIV-infected individuals.

Diabetes Mellitus and Other Comorbidities: In a study, Murray mentioned that type 2 diabetes most likely occurs in overweight or obese individuals, and there may be complex biological interactions between weight and diabetes, along with their impact on TB, because high body mass index (BMI) seems to protect against TB progression [6]. Hence, we need to adjust the BMI when interpreting data on the risk of TB disease progression among patients with diabetes [3]. A systematic review of 13 observational studies indicated that diabetes mellitus was associated with an increased risk of TB (relative risk = 3.11, 95% CI 2.27–4.26) [26].

Patients with end-stage renal disease have a high risk (20 times) of progression to TB than those without renal disease [27]. On the other hand, the risk of corticosteroid use for progression to active TB has been a controversial question [3]. There is an association between the use of TNF-α agents and progression to active TB among patients with autoimmune disorders, such as rheumatoid arthritis [28]. Patients with hematologic malignancies and head and neck cancer have a higher risk of progression to active TB disease than those in the general population [29].

Spontaneously Healed TB with Fibrotic Residuals
According to a 5-year follow-up observation in an untreated placebo group of an isoniazid prophylaxis control study, the incidence of active TB disease among those with fibrotic residuals on chest radiography was 2.9 per 1000 observation person-years [30]. Another 5-year follow-up of an isoniazid prophylaxis study conducted by the International Union Against Tuberculosis (IUAT) showed that the risk of developing active TB was twice as likely when the diameter of the fibrotic lesions on chest radiography was 2 cm or more compared to those with smaller-sized lesions [31].

Genetic Factors, Age, and Sex
A few studies conducted on twins have investigated the contribution of genetic factors toward active TB disease. In the early 1940s, Kallman et al. reported that hereditary factors influenced susceptibility to infection and the reaction of the host to the microorganism as monozygotic twins showed simultaneous occurrence and similarity in the course of primary TB more frequently as compared to dizygotic twins [32]. Some genetic polymorphisms in the host, such as the natural resistance-associated macrophage protein 1 (Nramp 1), have been suggested to be associated with active TB disease [3]. However, no statistically significant association was detected in a large-scale Hong Kong-Chinese study [33].

It might just be an effect of the cohort with the high rates of cases among the elderly merely due to high TB infection rates in the far past among them. Comstock *et al*. conducted a 20-year prospective study by recruiting more than 80,000 TST-positive children in a BCG vaccination trial in Puerto Rico. A remarkably high average annual incidence rate of 300–400 per 100,000 population was observed among children under 5 years of age. This number sharply dropped below 100, steadily rose to approximately 200 among young people, and then decreased to below 50 among those aged 25 years or older [34]. They also reported that the incidence of active TB was 18% higher among females than among males in TST-positive children [34].

Socio-Economic Factors
It has also been suggested that marital status contributes to active TB disease, wherein divorced males reportedly have a relatively higher risk of developing active TB disease than divorced females [35]. This might be due to the differences in social behaviors between males and females based on their marital status, which resulted in either lower or higher risk of MTB infection and subsequent disease frequency [3].

Poverty is attributed to a high incidence of TB within a community. This relationship might be owing to relatively congested living conditions that lead to an increased risk of MTB transmission and a high prevalence of infection with MTB, ultimately resulting in a high prevalence of TB in the community [3]. Similarly, migrants tend to stay or live under crowded, poorly ventilated conditions that facilitate the spread of MTB. With the high prevalence of MTB infection among them,

the prevalence of TB is also high. Furthermore, these people tend to have poor access to healthcare services, due to multiple possible reasons, such as economic, language, cultural, and information barriers, which result in late access to medical services and delayed treatment [3].

Body Mass Index
The pathological mechanisms underlying the increased risk of active TB disease among the low BMI people remain unknown. Tverdal showed that adults with a lower BMI in Norway had a relatively higher incidence of active TB disease, especially males [36]. Leung *et al*. in Hong Kong reported that "obese (BMI > 30) and overweight (BMI 25–30) individuals were protected from developing active TB with hazard ratios of 0.36 and 0.55, respectively, compared to those of normal weight" [37].

Tobacco Smoking and Indoor Air Pollution
Tobacco smoking causes the highest deaths related to noncommunicable diseases globally, while MTB is the biggest single agent causing deaths due to communicable diseases globally. Considering the global prevalence of tobacco smoking and TB, the combination of these two hazards should be attributable to enormous health risks. Even before 1960, Lowe and Edwards in England reported an association between tobacco smoking and pulmonary TB disease [38, 39]. In addition, passive smoking is recognized as one of the most common sources of indoor air pollution. It increases the risk of active TB disease among those exposed to tobacco smoke by approximately 5.4 times compared to those not exposed to tobacco smoke [40].

6 TB Deaths

6.1 TB Deaths in the Pre-Chemotherapy Era

First, we must bear in mind that confirming whether a death has been caused by TB, is often, if not always, challenging, for autopsy is rarely performed. There is no doubt that the confirming if a death was caused by TB was far from certain in the pre-chemotherapy era. Rieder summarized the cumulative TB case-fatality from untreated sputum smear-positive pulmonary TB in three European countries and indicated that approximately one-third of patients with sputum smear-positive TB died 1 year after diagnosis, and by 5 years, half to two-thirds of the patients had died [3].

6.2 Factors Related to TB Deaths in the Chemotherapy Era

After briefly describing that deaths among smear-positive pulmonary TB patients in the pre-chemotherapy era were common, we now raise the question as to why TB deaths occur in the current chemotherapy era.

6.2.1 TB Treatment Delays

TB diagnosis and treatment delays allow TB patients to spread MTB to several contacts, causing unfavorable outcomes, including death. Lienhart *et al*. showed that a TB diagnosis and treatment delay of more than 8 weeks was five times more likely to kill a patient, compared to those with shorter delays [41]. Delays in the diagnosis and treatment of TB also occur in economically developed and lower TB burden countries, such as Canada. Greenaway *et al*. reported that even in hospital settings in Canada, delays were observed in 30% of patients diagnosed with TB and were associated with a threefold increase in the risk of death [42]. Storla *et al*. conducted a systematic review of delays in TB diagnosis and treatment and reported three major factors contributing to this delay: socio-economic status, including low access to health care, rural residence, low education, and poverty; sociopsychological factors including initially seeking care from traditional healers, low-level government health facilities, and private practitioners; and demographic factors such as older age and female sex [43].

6.2.2 Comorbidities and Aging

It is well known that HIV infection enormously increases the risk of TB death and is the most substantial risk factor among other comorbidities. Duarte *et al*. estimated that the adjusted odds ratio of death among pulmonary TB patients with HIV infection was 11, as compared to those with no HIV infection [44]. They also shed light on the fact that renal failure, non-HIV-associated immunosuppression, malignancies, congestive heart failure, malnutrition, and alcoholism increased the risk of death among pulmonary TB patients. Aging in TB patients increases the risk of death due to various other reasons in addition to TB. Elderly patients with TB have a high rate of mortality, as observed in several countries in Asia. For instance, more than 32% of TB patients aged 65 years or more registered in 2018 died by the end of 2019 in Japan [45].

6.2.3 Failure to Complete TB Treatment and Drug Resistance

It is natural to expect that failure to complete the appropriate TB treatment even after TB diagnosis without delay causes unfavorable outcomes, including death. Kolapan *et al*. estimated the risk of death due to incomplete TB treatment to be 6.4 [46]. In India, Datta *et al*. reported that the risk of death increased according to a decrease in proportions of the prescribed anti-TB medicines that the patients consumed, and the trend increased with increasing age [47]. Incomplete TB treatment also increases the risk of drug resistance, which subsequently increases the risk of death. Kliiman *et al*. estimated that among those with previous TB treatment, multidrug-resistant TB (MDR TB) patients are approximately 2.4 times (20.2% vs. 8.3%) more likely to die compared with non-MDR TB patients [48].

7 The Molecular Epidemiology of TB

The molecular epidemiology of TB combines genotyping techniques of MTB with conventional epidemiologic methods to address questions related to MTB. It has been applied in a wide range of areas in regard to TB control and research; for instance, TB outbreak investigation, transmission of specific MTB strains in a population [49], identification of MTB strains with certain biological characteristics such as drug resistance, virulence, frequency of transmission, and clinical manifestations, detection of laboratory contamination, differentiation between relapse and reinfection among recurrent TB patients, and so forth [50]. Several genotyping methods have been developed and applied. For instance, restriction fragment length polymorphism (RFLP) targeting an insertion sequence (IS) *6110*, i.e., IS*6110*-RFLP, has become a standard method, followed by spoligotyping, variable numbers of tandem repeats (VNTR), and whole genome sequencing (WGS), a final stage of the genotyping method to reveal the complete sequence of MTB [51]. Owing to the exponential reduction in the cost of WGS along with the enormous technological advances in the past decades, it has become one of the standard tools to apply, particularly in many developed countries. As reported by Cohen *et al*., performing WGS on all newly detected MTB may help in better understanding the dynamics of MTB transmission, including drug-resistant strains [52].

8 The Impact of Coronavirus Disease 2019 (COVID-19) on TB Epidemiology

COVID-19, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), emerged in China at the end of 2019 and spread rapidly worldwide in merely a few months. As of May 20, 2021, COVID-19 has reportedly infected more than 588 million individuals and caused more than 6.4 million deaths, worldwide [53].

Although the main transmission route of SARS-CoV-2 is through exposure to respiratory droplets of an infectious virus, airborne transmission is also considered to be a route of infection, such as that of MTB [54]. The World Health Organization (WHO) and governments have been implementing social distancing measures, by ensuring that individuals avoid overcrowded situations, are in well-ventilated places, wear masks in indoor environments, and wash hands to mitigate the spread of SARS-CoV-2. These preventive measures may also reduce MTB transmission from infectious TB patients to contacts, thereby reducing the prevalence of infection with MTB in a community, subsequently reducing the prevalence of TB disease in the future.

The COVID-19 pandemic, in conjunction with “the stay at home” policy by national governments, lockdowns, collapse of medical service provision, and failure

of the supply chain of medical equipment, divert human resources and medical service system for TB control toward the COVID-19 response, and have led to hesitation from people to visit medical facilities even when they have respiratory symptoms suspecting of TB. This may cause a delay in TB diagnosis [55]. The WHO estimated that 1.4 million fewer people in the 84 countries received TB care in 2020, indicating a 21% reduction in TB case notifications from that in 2019 [56]. The WHO also estimated an increase of half a million TB deaths, meaning that COVID-19 would force the world to go back a decade in regard to TB mortality [56].

Visca reviewed TB and COVID-19 interactions and reported that a shared dysregulation of immune responses in COVID-19 and TB has been found, suggesting a dual risk posed by coinfection, worsening COVID-19 severity and favoring the disease progression of TB. He also mentioned that "COVID-19 can occur at any time during a patient's TB journey, with worse outcomes for patients affected by active pulmonary TB disease" [57].

9 The Global Burden of TB

9.1 Measurement of Disease Burden

TB is a notifiable disease in many countries around the globe. The compiled data are reported to the WHO annually. Many countries, including developing countries, have introduced electronic surveillance systems, which make it possible to acquire almost real-time data from the field [58]. However, it is not easy to measure the correct burden of disease from the notification data because it is quite often the case that the gap between notification and actual incidence of a disease is substantial. The possible causes of this difference are undiagnosed cases due to barriers to access to health care, poor health-seeking behavior, poor quality of diagnosis, and diagnosed but unnotified cases due to problems in the reporting system (Fig. 1.6). Social determinants for access to medical care are poverty, stigma, low education, economic barriers, physical access (distance or transportation to the health facility), health insurance, health resources, social welfare services, and social protection [59].

Many interventions are required to minimize this gap. The countermeasures for better access to diagnosis include active case finding, the establishment of universal health coverage (UHC) including health insurance, development of health infrastructure, and so forth. Civil society also definitely has significant roles in changing the health-seeking behavior of the community. For better diagnostic services, the introduction of quality assurance and sensitive diagnostic tools should be considered. To minimize diagnosed but unnotified cases, public–private partnership for national TB control programs is recommended [60]. To enforce medical facilities to notify cases, financial incentives or penalties may work.

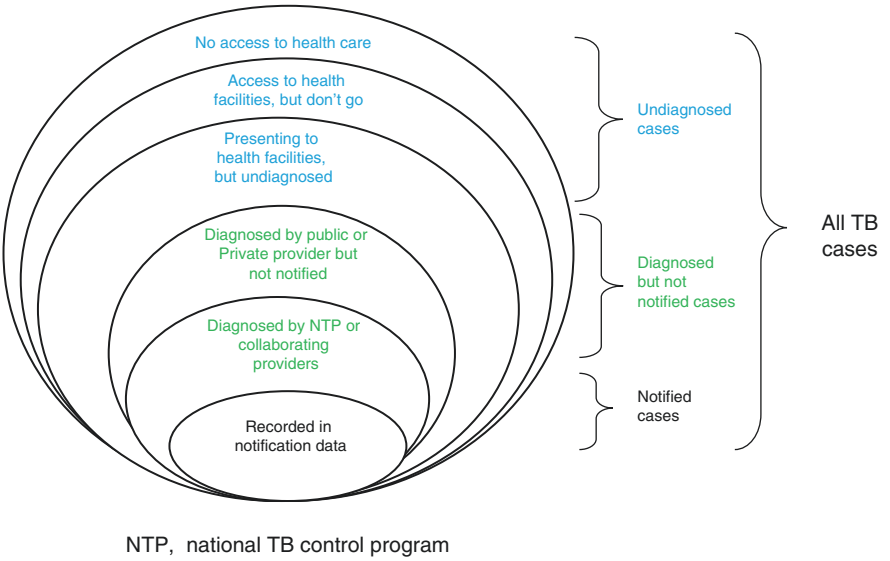


Fig. 1.6 The "Onion" model: a framework for assessing the fraction of TB cases accounted for in TB notification data [59]

A TB prevalence survey is a direct methodology to evaluate existing TB cases in a certain area. In the survey, methodology and quality assurance make difference in sensitivity and specificity. The report of the prevalence survey was published by the WHO [61].

In recent TB prevalence surveys, all participants were requested to take chest radiographs, which revealed a certain number of asymptomatic active TB cases. The estimated incidence increased in countries where prevalence surveys were conducted using updated procedures. This is not due to the deterioration of TB control but due to the improvement of accuracy of the survey, which was reflected in the estimation of the burden [61].

9.2 The Historical View of TB Epidemiology

Detecting TB in humans can be traced back to the signs of caries on the bones of a mummy of the ancient Egyptian dynasty era. During the eighteenth century, the industrial revolution and urbanization induced an epidemic of TB in Western countries. Congestive and poor working environments, long working hours, and poor nutrition status due to poverty were the causes of the spread of the disease. Because of its extremely high mortality, TB was called "White plague." TB patients had to place their hopes on sanatorium therapy, which was composed of fresh air, nutrition, and good rest [62].

The number of TB patients started to decrease early in the twentieth century in Western countries, most likely due to the improvement in social conditions. For instance, legislation on social welfare, such as the Factory Act, contributed to a decrease in TB in the UK. Modern chemotherapy, which was introduced after World War II, significantly accelerated the decline in TB burden. In the 1970s, TB was thought to be a disease of the past in some Western countries, and funding for TB control was markedly reduced. However, a resurgence of TB occurred from the 1980s till the early 1990s in many parts of industrialized countries. Factors related to the resurgence were spread of HIV, an increase in drug-resistant TB, population migration, and the prevalence of noncommunicable diseases affecting TB, such as DM, potentially dismantling the TB control program [63, 64].

The history of TB control in Japan is unique. The industrial revolution, which started in the late nineteenth century and the series of military confrontations and wars in the mid-twentieth century caused an explosive increase in TB. TB was then called "a nation's disease" as it was the number one killer among all causes of death. The TB Control Law, which was revised in 1951, included virtually all components of TB control strategies, such as intensive active case finding with chest radiography, public–private partnership, management of the quality of medical service by checking treatment regimens at local public health centers, patient-centered support for patient adherence to anti-TB medications by public health centers, and BCG vaccination provided to all children. The establishment of a National Health Insurance scheme in 1961 accelerated early case finding of symptomatic patients through the improvement of access to medical services. With all these efforts, Japan achieved an annual decline of 11% in TB incidence before 1980, which is equivalent to the current target of the End TB Strategy, between 2015 and 2025 (Fig. 1.7) [65].

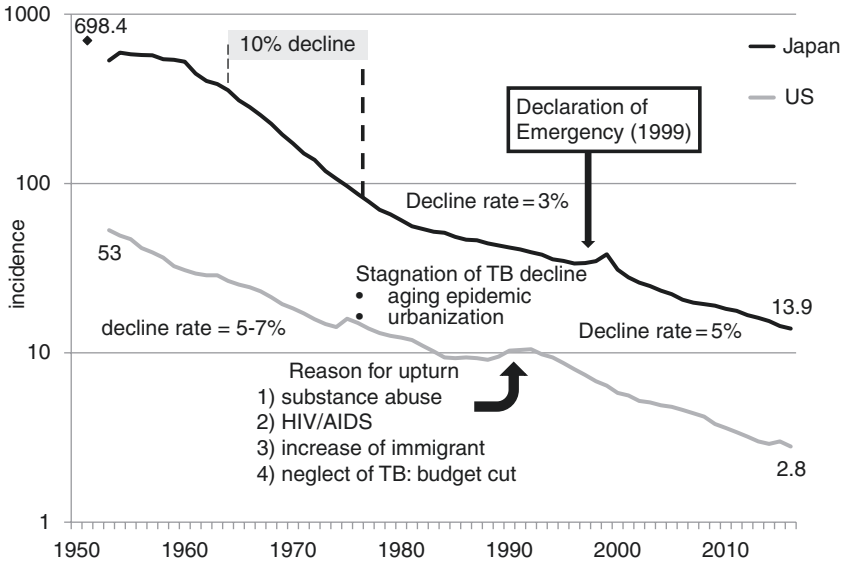


Fig. 1.7 Trend of tuberculosis incidence in the USA and Japan

9.3 Current TB Epidemiology

According to the Global TB Report 2020 by the WHO, the estimated number of incident TB patients in 2019 was 10.0 million (range, 8.9–11.0 million) [58]. Men (aged ≥15 years), women (aged ≥15 years), and children (aged<15 years) accounted for 56%, 34%, and 12% of all TB patients, respectively. It is estimated that TB deaths among HIV-negative people were 1.2 million, and an additional 208,000 deaths (range, 177,000–242,000) were reported among HIV-positive individuals [58]. The geographical distribution by the WHO regions from top to bottom was Southeast Asia, Africa, Western Pacific, Eastern Mediterranean, America, and Europe, with proportions of 44%, 25%, 18%, 8.2%, 2.9%, and 2.5%, respectively. The age and sex distribution of the estimated incidence and notification were quite diverse according to the WHO region. The highest incidence based on age groups in males were 45–54, 25–34, ≥65, ≥65, 25–34, and 35–45, in Southeast Asia, Africa, Western Pacific, Eastern Mediterranean, America, and Europe. Eight countries accounted for two-thirds of all TB patients combined: India (26%), Indonesia (8.5%), China (8.4%), the Philippines (6.0%), Pakistan (5.7%), Nigeria (4.4%), Bangladesh (3.6%), and South Africa (3.6%). The diversity of the gap in age and sex distribution was also observed at the country level. In Japan, two-thirds of TB patients were over 65 years old and half were over 75 years old. It is because aged TB patients develop the disease mostly due to the recurrence of past infection, which may have occurred during the era of the TB endemic. The other possible reason is an extremely high-aged society due to its low birth rate and longevity. The aging of TB patients is observed across Asian countries and will be a global issue of TB control in the future [58].

A total of 47 countries are low TB incidence, i.e., incidence rates of less than 10 per 100,000 population, as of 2019. Most of them are in Western Europe and North America, while a few are in the Western Pacific and Eastern Mediterranean regions. In these low-incidence countries, native TB patients are mostly older, who develop the disease as reactivation from long past infection, when the risk of infection was much higher than it is now. Instead, foreign-born patients comprise more than half of all patients in many countries. The major concerns of global TB epidemiology are discussed in the following sections.

9.3.1 HIV/TB

The risk of developing TB among people living with HIV was 18 times (range, 15–21) higher than that in the rest of the global population. The incidence of TB expressed per 100 person-years with HIV was 2.1% (range, 1.9–2.4%) [58]. HIV was the most prominent risk factor for an increase in TB incidence. Antiretroviral therapy (ART) for HIV is effective in controlling viral load and maintaining the immune system. The introduction of ART has drastically reduced the rate of mortality and incidence of HIV/TB in the past decades. In 2019, the estimated number of

HIV/TB cases comprised 8.2% of all TB patients; however, the reported case number was only 56% of the estimated number. The proportion of TB cases coinfected with HIV was very high in countries in the WHO African region. Some countries in southern Africa exceeded 50% of the coinfection rate. The proportion of TB patients with known HIV status was 71% globally and 86% in Africa. Thus, the situation of HIV/TB has greatly improved over the last two decades [58].

9.3.2 Multidrug-Resistant TB

MDR TB is defined as TB that is resistant to rifampicin and isoniazid. MDR TB is the result of improper treatment with improper regimens and/or irregular drug administration. However, a certain proportion of patients with MDR TB are newly treated cases, implying that such patients got infected from MDR TB patients.

MDR TB remains a major public health concern. In recent years, GeneXpert, which can detect rifampicin-resistant *M. tuberculosis* strains more easily than before, has been widely used in resource-limited countries. It is known that 78% of rifampicin resistance (RR) MTB results by GeneXpert appear to be MDR [58]. According to the WHO Global TB Report 2020, 3.3% (95% confidence interval [CI]: 2.3–4.3%) of new cases and 18% (95% CI: 9.7–27%) of previously treated cases had MDR/RR-TB. The proportion of TB with MDR/RR was very high (>20% in new cases and > 50% in previously treated cases) in several countries of the former Soviet Union [58].

9.3.3 Migrants

In most low-incidence countries where TB notification rates of native-born patients continue to decrease, the proportion of foreign-born TB patients from TB endemic countries has been increasing up to 60–90% in recent years [66]. Foreign-born TB patients are young, as they come to the country to study or seeking jobs. The proportion of MDR TB among foreign-born TB patients is generally high, reflecting that of the country of origin. The transmission from migrants to the native population is often modest. TB notification among migrants depends on the number and profile of migrants, as well as the TB control strategy for migrants [67].

In countries where urbanization is progressing, such as China, internal migrants from rural areas constitute a high-risk population living in socially depressed areas of big cities [68].

9.3.4 Elderly Population

In many low-TB burden settings, population aging has progressed over the past few decades. In such countries, the burden of TB has shifted from young age groups toward older age groups, at least in the non-foreign-born population. Aging of TB

patients may be caused by (1) a rapid decline in TB incidence in the young generation, (2) a decrease in the young generation due to low birth rate or military battles, and (3) longevity of the population. It has started to occur in some developing countries and will be a major challenge to global TB control in the future.

9.3.5 Global TB Strategy by the WHO

The DOTS Strategy

Despite the heavy disease burden, TB was a neglected disease at the global level until the 1980s. In 1993, the WHO declared a "Global TB Emergency" to address the urgent need to strengthen TB control. At the World Health Assembly 1994, the WHO adopted the DOTS Strategy, which was composed of five elements: (1) Government commitment, (2) Case detection by sputum smear microscopic examination through predominantly passive case finding, (3) Standardized short-course chemotherapy for at least all confirmed smear-positive TB under proper case management conditions and DOT during the entire course of treatment, (4) Establishment of a system for regular, uninterrupted supply of all essential anti-TB drugs, and (5) Establishment and maintenance of a monitoring system for both program supervision and evaluation. The DOTS Strategy was considered to be one of the most cost-effective public health interventions for reducing mortality and transmission [69].

9.3.6 The Stop TB Strategy

Following the DOTS Strategy, the WHO implemented a Stop TB Strategy between 2006 and 2015, which was in line with the Millennium Development Goals (MDGs). The strategy was composed of six key elements: (1) Pursue quality DOTS expansion and enhancement, improving case finding and cure through an effective patient-centered approach to reach all patients, especially the poor; (2) Address TB/HIV, MDR TB, and other challenges by scaling up TB/HIV joint activities, DOTS-Plus, and other relevant approaches; (3) Contributing to health system strengthening by collaborating with other health programs and general services, for instance, mobilizing the necessary human and financial resources for implementation and impact evaluation, and in sharing and applying achievements of TB control; (4) Involve all care providers, i.e., public, nongovernmental, and private organizations, by scaling up approaches based on a public-private mix (PPM), to ensure adherence to the International Standards of TB Care; (5) Engage people with TB and affected communities to demand and contribute to effective care; and (6) Enable and promote research for the development of new drugs and diagnostic strategies. Research is also needed to improve program performance [70]. The efforts made by high-burden countries contributed greatly to meet the TB-related target of the MDGs of halting and beginning to reverse the TB epidemic; however, it had very little effect on

achieving the desired impact in terms of decreasing the incidence rates and driving down the TB epidemic.

9.3.7 The End TB Strategy

At the World Health Assembly in 2014, the WHO approved the End TB Strategy, which envisions universal access to high-quality TB care and to promote TB prevention [71]. It is designed to achieve a health-related target under the United Nations Sustainable Development Goals (SDGs) 3, which calls for ending the global TB epidemic. The targets of the strategy are very ambitious, that include: (1) reducing the TB incidence rate by 90% to ≤10 cases per 100,000 population per year by 2035 compared with a baseline of 2015; (2) reducing the absolute number of TB deaths by 95% by 2035 compared with a baseline of 2015; and (3) eliminating catastrophic costs faced by TB-affected families to be achieved by 2020. The pillars and components are as follows: (1) integrated, patient-centered care and prevention, including (a) early diagnosis of TB, including universal drug-susceptibility testing and systematic screening of contacts and high-risk groups, (b) treatment of all individuals with TB, including drug-resistant TB, and patient support, (c) collaborative TB/HIV activities, and management of comorbidities, (d) preventive treatment of persons at high risk, and vaccination against TB; (2) bold policies and supportive system, including (a) political commitment with adequate resources for TB care and prevention, (b) engagement of communities, civil society organizations, and public and private care providers, (c) universal health coverage policy, and regulatory frameworks for case notification, vital registration, quality and rational use of medicines, and infection control, (d) social protection, poverty alleviation, and actions on other determinants of TB; (3) intensified research and innovation, including (a) discovery, development, and rapid uptake of new tools, interventions, and strategies, (b) research to optimize implementation and impact, and promote innovations.

In line with the End TB Strategy, the WHO and European Respiratory Society created a framework for TB elimination in countries with low incidence [72]. It outlines priority action areas for accelerating progress toward pre-elimination by 2035 and elimination of TB by or before 2050. It requires better access to high-quality diagnosis and care and more effective TB prevention, including addressing the social determinants of TB, with special attention to groups at the highest risk for TB with the introduction of new tools such as potential vaccines [72].

10 Conclusion

Information on global TB epidemiology is important for clinical practice because TB is associated with social determinants. Social determinants for risk of TB infection are as follows: poverty, unstable diet, poor living conditions, air pollution, working environment, housing, congregate setting, homelessness, prison,

and so forth. Individual risk factors for developing active TB include HIV/AIDS, DM, hemodialysis, smoking, malnutrition, immunosuppression, and mental illness.

It is also important for clinicians to understand that the early diagnosis of TB, proper treatment with standardized regimens, and patient-centered support for adherence are prerequisites for reducing the burden of TB.

References

1. Rasanathan K, Sivasankara Kurup A, Jaramillo E, Lönnroth K. The social determinants of health: key to global tuberculosis control. Int J Tuberc Lung Dis. 2011;15(6):S30–6. https://doi.org/10.5588/ijtld.10.0691.
2. Murray M. The epidemiology of tuberculosis. In: Raviglione MC edited. Informa health care, tuberculosis fourth ed. the essentials. New York: Informa Healthcare USA, Inc.; 2010. p. 23–59.
3. Rieder HL. Epidemiologic basis of tuberculosis control. Paris: International Union Against TB and Lung Disease; 2003.
4. Aoki M. Transmission of tuberculosis (I). Kekkaku. 2004;79:509–18. [in Japanese]
5. Pai M, Riley LW, Colford JM Jr. Interferon-γ assays in the immunodiagnosis of tuberculosis: a systematic review. Lancet Infect Dis. 2004;4:761–76.
6. Andersen P, Munk ME, Pollock JM, Doherty TM. Specific immune-based diagnosis of tuberculosis. Lancet. 2000;356:1099–104.
7. Styblo K. Epidemiology of tuberculosis. In: The Hague: Royal Netherlands Tuberculosis Association. 2nd ed; 1991.
8. Styblo K. The relationship between the risk of tuberculous infection and the risk of developing infectious tuberculosis. Bull Int Union Tuberc. 1985;60:117–9.
9. Cauthen GM, Rieder HL, Geiter LJ. A model of the relation between age-specific prevalence of tuberculous infection and incidence of infectious tuberculosis: implications for screening policies. Tuberculosis Surveillance Research Unit Progress Report. 1991;1:1–20.
10. Riley RL. The hazard is relative. (editorial). Am Rev Respir Dis. 1967;96:623–5.
11. Snider DE Jr, Kelly GD, Cauthen GM, et al. Infection and disease among contacts of tuberculosis cases with drug-resistant and drug-susceptible bacilli. Am Rev Respir Dis. 1985;132:125–32.
12. Tostmann A, Kik SV, Kalisvaart NA, Sebek MM, Verver S, Boeree MJ, van Soolingen D. Tuberculosis transmission by patients with smear-negative pulmonary tuberculosis in a large cohort in the Netherlands. Clin Infect Dis. 2008;47:1135–42.
13. Rouillon A, Perdrizet S, Parrot R. Transmission of tubercle bacilli: the effects of chemotherapy. Tubercle. 1976;57:275–99.
14. Rogers EFH. Epidemiology of an outbreak of tuberculosis among school children. Public Health Report. 1962;77:401–6.
15. Catanzaro A. Nosocomial tuberculosis. Am Rev Resp Dis. 1982;125:559–62.
16. National Tuberculosis Institute. Tuberculosis in a rural population of South India: a five-year epidemiological study. Bull World Health Organ. 1974;51:473–88.
17. Aoki M. Kekkakubyougaku no Tenbo. Kekkaku. 1983;58:371–8. [in Japanese]
18. Wallgren A. Time-table of tuberculosis. Tubercle. 1948;29:245–51.
19. Chiba Y. Significance of Endogeneous reactivation 30 year follow-up of tuberculin positive converters. Bull IUAT. 1974;49:321–4.
20. Grzybowski S, Barnett CD, Styblo K. Contact of cases of active pulmonary tuberculosis. Bull IUAT. 1975;50:90–106.

21. de Jong BC, Hill PC, Aiken A, Awine T, Antonio M, Adetifa IM, Jackson-Sillah DJ, et al. Progression to active tuberculosis, but not transmission, varies by mycobacterium tuberculosis lineage in the Gambia. J Infect Dis. 2008;198:1037–43.
22. Kamat SR, Dawson JJY, Devadatta S, Fox W, Janardhanam B, Radhakrishna S, et al. A controlled study of the influence of segregation of tuberculous patients for one year on the attack rate of tuberculosis for a 5-year period in close family contacts in South India. Bull World Health Organ. 1966;34:517–32.
23. Grzybowski S, Galbraith JD, Styblo K, Chan-Yeung M, Dorken E, Brown A. Tuberculosis in Canadian Eskimos. Arch Environ Health. 1972;25:329–32.
24. Sutherland I. The epidemiology of tuberculosis and AIDS. British Communicable Disease Report. 1990;90(10):3–4.
25. Selwyn PA, Hartel D, Lewis VA, Schoenbaum EE, Vermund SH, Klein RS, et al. A prospective study of the risk of tuberculosis among young intravenous drug users with human immunodeficiency virus infection. N Engl J Med. 1989;320:545–50.
26. Jeon CY, Murray MB. Diabetes mellitus increases the risk of active tuberculosis: a systematic review of 13 observational studies. PLoS Med. 2008;5(7):e152.
27. Andrew OT, Schoenfeld PY, Hopewell PC, Humphreys MH. Tuberculosis in patients with end-stage renal disease. Am J Med. 1980;68:59–65.
28. Gardam MA, Keystone EC, Menzies R, Manners S, Skamene E, Long R, et al. Anti-tumour necrosis factor agents and tuberculosis risk: mechanisms of action and clinical management. Lancet Infect Dis. 2003;3:148–55.
29. Kamboj M, Sepkowitz KA. The risk of tuberculosis in patients with cancer. Clin Infect Dis. 2006;42:1592–5.
30. Falk A, Fuchs GF. Prophylaxis with isoniazid in inactive tuberculosis. A veterans administration cooperative study XII. Chest. 1978;73:44–8.
31. International Union Against Tuberculosis Committee on Prophylaxis. Efficacy of various durations of isoniazid preventive therapy for tuberculosis: five years of follow-up in the IUAT trial. Bull World Health Organ. 1982;60:555–64.
32. Kallmann F, Reisner D. Twin studies on the significance of genetic factors in tuberculosis. Am Rev Tuberc. 1943;47:549–74.
33. Li HT, Zhang TT, Zhou YQ, Huang QH, Huang J. SLC11Al (formerly NRAMPI) gene polymorphisms and tuberculosis susceptibility: a meta-analysis. lnt J Tuberc Lung Dis. 2006;10:3–12.
34. Comstock GW, Livesay VT, Woolpert SF. The prognosis of a positive tuberculin reaction in childhood and adolescence. Am J Epidemiol. 1974;99:131–8.
35. Horwitz O. Tuberculosis risk and marital status. Am Rev Respir Dis. 1971;104:22–31.
36. Tverdal A. Body mass index and incidence of tuberculosis. Eur J Respir Dis. 1986;69:355–62.
37. Leung CC, Lam TH, Chan WM, Yew WW, Ho KS, Leung G, et al. Lower risk of tuberculosis in obesity. Arch Intern Med. 2007;167:1297–304.
38. Lowe CR. An association between smoking and respiratory tuberculosis. Br Med J. 1956;2:1081–6.
39. Edwards JH. Contribution of cigarette smoking to respiratory disease. Br J Prev Soc Med. 1957;11:10–21.
40. Lin HH, Ezzati M, Murray M. Tobacco smoke, indoor air pollution and tuberculosis: a systematic review and meta-analysis. PLoS Med. 2007;4:e20.
41. Lienhardt C, Rowley J, Mannneh K, Lahai G, Needham D, Milligan P, et al. Factors affecting time delay to treatment in a tuberculosis control programme in a sub-Saharan African country: the experience of the Gambia. lnt J Tuberc Lung Dis. 2001;5:233–9.
42. Greenaway C, Menzies D, Fanning A, Grewal R, Yuan L, FitzGerald JM, et al. Delay in diagnosis among hospitalized patients with active tuberculosis–predictors and outcomes. Am J Respir Crit Care Med. 2002;165:927–33.
43. Storla DG, Yimer S, Bjune GA. A systematic review of delay in the diagnosis and treatment of tuberculosis. BMC Public Health. 2008;8:15.

44. Duarte EC, Bierrenbach AL, da Silva B, Junior J Jr, Tauil PL, de Fátima DE. Factors associated with deaths among pulmonary tuberculosis patients: a case-control study with secondary data. J Epidemiol Community Health. 2009;63:233–8.
45. Tuberculosis Surveillance Center. Tuberculosis in Japan–annual report 2020. Tokyo: Department of Epidemiology and Clinical Research, Research Institute of Tuberculosis; 2020.
46. Kolappan C, Subramani R, Kumaraswami V, Santha T, Narayanan PR. Excess mortality and risk factors for mortality among a cohort of TB patients from rural South India. Int J Tuberc Lung Dis. 2008;12:81–6.
47. Datta M, Radhamani MP, Selvaraj R, Paramasivan CN, Gopalan BN, Sudeendra CR, et al. Critical assessment of smear-positive pulmonary tuberculosis patients after chemotherapy under the direct tuberculosis programme. Tubercle Lung Dis. 1993;74:180–6.
48. Kliiman K, Altraja A. Predictors of poor treatment outcome in highly drug-resistant pulmonary tuberculosis. Eur Respir J. 2009;33:1085–94.
49. Couvin D, David A, Zozio T, Rastogi N. Macro-geographical specificities of the prevailing tuberculosis epidemic as seen through SITVIT2, an updated version of the mycobacterium tuberculosis genotyping database. Infect Genet Evol. 2019;72:31–43.
50. Mathema B, Kurepina NE, Bifani PJ, Kreiswirth BN. Molecular epidemiology of tuberculosis: current insights. Clin Microbiol Rev. 2006;19:658–85.
51. Jagielski T, Minias A, van Ingen J, Rastogi N, Brzostek A, Żaczek A, et al. Methodological and clinical aspects of the molecular epidemiology of mycobacterium tuberculosis and other mycobacteria. Clin Microbiol Rev. 2016;29:239–90.
52. Cohen KA, Manson AL, Abeel T, Desjardins CA, Chapman SB, Hoffner S, et al. Extensive global movement of multidrug-resistant M. tuberculosis strains revealed by whole-genome analysis. Thorax. 2019;74:882–9. https://doi.org/10.1136/thoraxjnl-2018-211616.
53. COVID-19 Dashboard, Coronavirus resource center, Johns Hopkins University. https://coronavirus.jhu.edu/map.html. Accessed 12 August, 2022.
54. Morawska L, Milton DK. It is time to address airborne transmission of coronavirus disease 2019 (COVID-19). Clin Infect Dis. 2020;71:2311–3.
55. Narita M, Hatt G, Toren KG, Vuong K, Pecha M, Jereb JA, et al. Delayed tuberculosis diagnoses during the COVID-19 pandemic in 2020–King County, Washington. Clin Infect Dis 2021;ciab387.
56. WHO. Impact of the COVID-19 pandemic on TB detection and mortality in 2020. https://www.who.int/publications/m/item/impact-of-the-covid-19-pandemic-on-tb-detection-and-mortality-in-2020. Accessed 20 May, 2021.
57. Visca D. Tuberculosis and COVID-19 interaction: a review of biological, clinical and public health effects. Pulmonology. 2021;27:151–65.
58. Global tuberculosis report 2020. Geneva: World Health Organization; 2020.
59. Early detection of Tuberculosis. An overview of approaches, guidelines and tools. Geneva: World Health Organization; 2011. (WHO/HTM/STB/PSI/2011.21)
60. Engaging private health care providers in TB care and prevention: a landscape analysis, 2020 update. Geneva: World Health Organization; 2021. Licence: CC BY-NC-SA 3.0 IGO.
61. National tuberculosis prevalence surveys 2007–2016. Geneva: World Health Organization; 2021. Licence: CC BY-NC-SA 3.0 IGO.
62. Dubos RJ, Dubos J. The white plague: tuberculosis, man, and society. Rutgers University Press; 1952.
63. Glaziou P, Floyd K, Raviglione M. Trends in tuberculosis in the UK. Thorax. 2018;73:702–3.
64. Committee on the Elimination of Tuberculosis in the United State. Ending Neglect. Institute of Medicine, National Academy Press, Washington, D.C., 2000.
65. Aoki M. Characteristics of tuberculosis in Japan compared to those in other developed countries. Kekkaku. 1993;68:533–8. [in Japanese]
66. European Centre for Disease Prevention and Control, WHO Regional Office for Europe. Tuberculosis surveillance and monitoring in Europe 2021–2019 data.

67. Lonnroth K, Mor Z, Erkens C, et al. Tuberculosis in migrants in low-incidence countries: epidemiology and intervention entry points. Int J Tubercl Lung Dis. 2018;21:624–36.
68. Jia ZW, Jia XW, Liu YX, et al. Spatial analysis of tuberculosis cases in migrants and permanent residents, Beijing, 2000–2006. Emerg Infect Dis. 2008;14:1413–9.
69. Borgdorff MW, Floyd K, Broekmans JF. Interventions to reduce tuberculosis mortality and transmission in low- and middle-income countries. Bulletin of the World Health Org. 2002;80:217–27.
70. The Stop TB Strategy. World Health Organization 2006, WHO/HTM/STB/2006.37.
71. Implementing the end TB strategy: the essentials. ISBN 978 92 4 150993 0 (NLM classification: WF 200).
72. Towards tuberculosis elimination: an action framework for low-incidence countries. World Health Organization the European Respiratory Society 2014 ISBN 978 92 4 150770 7 (NLM classification: WF 200) WHO/HTM/TB/2014.13.

Chapter 2
Immunology: How Does the Immune System Affect the Development of Pulmonary TB? How Does an Understanding of TB Immunology Help Clinicians Manage their Patients with Pulmonary TB?

Masashi Matsuyama and Yukio Ishii

Abstract TB is the leading cause of death from a single infectious agent. *Mycobacterium tuberculosis*—the causative agent of TB—is an intracellular pathogen that is transmitted through the air. When tuberculous bacilli first enter the lung, innate and acquired immune responses are induced. The fact that experimental TB cavities can be formed by the administration of dead *Mycobacterium tuberculosis* alone indicates how important TB immunity is in the formation of TB lesions. TB bacteria have a variety of defense mechanisms that allow them to survive within macrophages. Thus, low exposure to *Mycobacterium tuberculosis* is bactericidal, but high exposure can lead to the development of TB and latent infection. The IFN-γ/IL-12 axis—a positive feedback mechanism between macrophages and Th1 cells—is indispensable for the control of TB infection. Recently, TNF-α inhibitors and PD-1 pathway inhibitors have been frequently used in clinical practice. However, recent studies reported that these drugs were associated with the exacerbation of TB infection. Therefore, it is important for clinicians to understand TB immunity.

Keywords Macrophages · T cells · Pattern recognition receptors · IFN-γ/IL-12 axis · TNFα · PD-1 pathway

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M. Matsuyama · Y. Ishii (✉)
Department of Respiratory Medicine, University of Tsukuba, Ibaraki, Japan
e-mail: ishii-y@md.tsukuba.ac.jp

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1 Introduction

TB is the leading cause of death from a single infectious pathogen. Globally, an estimated ten million people fell ill with TB and 1.4 million people died from TB in 2019. *Mycobacterium tuberculosis*, which is the causative agent of TB, is an intracellular pathogen that is transmitted through the air. In the general population, however, most infected individuals with *M. tuberculosis* never develop clinically active TB disease [1].

TB infection occurs following the inhalation of *M. tuberculosis* bacilli-containing aerosolized droplets. These bacilli are then phagocytosed by alveolar macrophages in the alveolar spaces. Macrophages can adequately control the sterilization of *M. tuberculosis* when exposure to the bacteria is low. However, *M. tuberculosis* proliferates in these cells during the early stage of infection when individuals are exposed to high levels of TB bacteria. Thereafter, an adaptive immune response to TB is induced and most infected people avoid developing the disease. However, 5%–10% of infected individuals whose immune response is insufficient develop TB disease [1]. In addition, the immune system of some infected individuals does not completely eliminate *M. tuberculosis* from the body, but rather maintains the TB infection, which is termed latent TB infection (LTBI) (Fig. 2.1). Furthermore, individuals who have complications related to diminished immune functions, easily develop active TB from LTBI. These findings demonstrate that host immune responses are important for controlling the development of active TB disease.

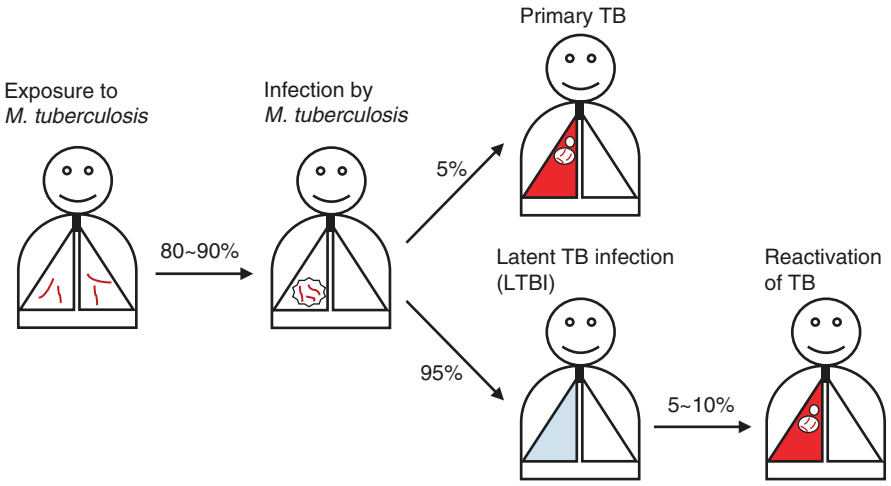

Fig. 2.1 Natural history of human TB infection. Approximately 10%–20% of individuals do not become infected and never develop disease even if they are exposed to high numbers of TB bacilli. Approximately 5% of infected individuals develop clinical TB disease within 2 years of infection. The remaining infected individuals do not develop active TB disease but maintain a TB infection, termed latent tuberculosis infection (LTBI). Approximately 5%–10% of LTBI subjects develop clinical TB during their lifetime by reactivation of the original infection related to a decline of T cell immunity

In many species, including humans, the immune system is divided into two major subsystems: innate immunity and acquired immunity. Coordination between innate and acquired immunity is needed to generate host resistance to TB infection. Because *M. tuberculosis* is an intracellular bacterium, antibodies are not useful for protective immunity. Rather, cellular immunity by IFN-γ-producing cytotoxic T cells is the mainstay of TB immunity.

Interestingly, the formation of experimental tuberculous cavities does not necessarily require live bacteria but depends on the host's immune response to *M. tuberculosis* protein antigens [2]. Furthermore, one study has shown that a delayed allergic reaction to the protein antigen of *M. tuberculosis* is involved in the formation of tuberculous cavities [2]. TB complicated with human immunodeficiency virus (HIV) infection, in which the number of CD4-positive T cells is markedly reduced, does not develop typical cavity formation [3]. These findings indicate that the development of TB is closely related to host immune conditions rather than the virulence of mycobacteria.

In this chapter, TB immunity is reviewed in terms of innate and acquired immunity. In addition, the mechanism of TB development under immunosuppressive conditions will be explained. Recently, PD-1 pathway inhibitors that activate T-cell immunity have been used for cancer treatment. Finally, we review the relationship between TB exacerbation and PD-1 pathway inhibitors.

2 Innate Immunity against *M. Tuberculosis*

When *M. tuberculosis* bacilli enter into alveolar spaces, they first encounter innate immune cells, such as alveolar macrophages and dendritic cells (DCs). In high TB epidemic areas, half of exposed people never get infected with *M. tuberculosis* and remain negative for the tuberculin skin test and IFN-γ release test [4]. Only the activation of Toll-like receptor (TLR)-2 expressed on human and mouse macrophages by microbial lipoproteins has been shown to kill intracellular *M. tuberculosis*, providing direct evidence for the innate immune-mediated clearance of *M. tuberculosis* [5]. Thus, innate immune responses are associated with the early clearance of *M. tuberculosis* before the onset of adaptive immunity. However, when the number of bacteria is high, innate immunity induces acquired immune responses to control the infection because sterilization by innate immunity alone is insufficient. Moreover, innate immune cells are often a niche for the replication of *M. tuberculosis*, which uses a variety of strategies to subvert the innate immune response.

2.1 Recognition of M. Tuberculosis *by Pattern Recognition Receptors*

Pathogen-associated molecular patterns (PAMPs) on *M. tuberculosis* are recognized by pattern recognition receptors (PRRs). Innate immune cells express various PRRs, including TLRs (e.g., TLR-2, TLR-4, and TLR-9), Nod-like receptors (e.g., NOD1,

NOD2, NLRP3, and NLRC4), absent in melanoma 2 (AIM2)-like receptors, C-type lectins (e.g., Mannose receptors, DC-SIGN, Dectin-1, Dectin-2, and Mincle), complement receptors (e.g., Complement receptor 3), collectins (e.g., Surfactant proteins A and D, Mannose-binding lectin), scavenger receptors (e.g., MARCO, SR-A1, and CD36, SR-B1), Fc receptors (e.g., FcgR), and glycophosphatidylinositol-anchored membrane receptors (e.g., CD14) (Fig. 2.2) [6–8]. Mannosylated lipoarabinomannan (ManLAM), phosphatidyl-inositol mannoside (PIM), phthiocerol dimycocerosates (PDIMs), phenolic glycolipids (PGLs), trehalose dimycolate (TDM), peptidoglycans, and other mycobacterial components are recognized by an array of cell surface and intracellular PRRs that mediate phagocytosis and/or antimicrobial defense systems. *M. tuberculosis* DNA [9, 10] or bacterial second messengers [11] can be recognized by cytosolic PRRs, such as cGAS and STING [12, 13], which induce downstream cytokine production and autophagy.

Regarding TLRs, *M. tuberculosis* expresses a variety of known or putative TLR ligands and TLR-2, TLR-4, and TLR-9 have been implicated in the host recognition of *M. tuberculosis* [6, 8]. The contribution of individual TLRs to immunity against *M. tuberculosis* infection varies, but the importance of the TLR signaling pathway for antimycobacterial immunity is evident in studies showing that mice lacking the common TLR adaptor protein, myeloid differentiation factor 88 (MyD88), quickly succumb to *M. tuberculosis* infection [14, 15]. The susceptibility of MyD88-knockout mice to *M. tuberculosis* infection has been attributed to the deficient expression of NOS2 [15], an impaired ability to activate the IL-1β or IL-1 receptor (IL1R) pathway [16, 17], impaired receptivity of macrophages to IFN-γ signaling [18], and impaired IL-12 and TNF-α responses in macrophages and DCs [14]. In addition, polymorphisms in specific TLRs or TLR signaling proteins have also been strongly associated with pulmonary TB in humans or have been shown to influence immunity against *M. tuberculosis* [19–22].

While the host recognition of *M. tuberculosis* leads to the activation of innate immunity, *M. tuberculosis* has also evolved strategies that allow it to evade innate immune responses mediated by PRRs. The strain-specific expression of cell envelope components may be associated with differential immune responses. For example, the W-Beijing lineage strain, HN878, expresses polyketide synthase-derived PGLs that are missing in laboratory-adapted H37Rv, or other clinical isolates (i.e., CDC1551) [23]. The expression of PGL by HN878 reduced the production of multiple innate immune cytokines and chemokines in host cells [23, 24], although its role in the increased virulence of HN878 remains controversial. The modulation of innate immune responses by *M. tuberculosis* is mediated by the presence of immune inhibitory lipid components that compete with immune-activating mycobacterial components for the same receptors. For example, the expression of tetraacylated sulfoglycolipids in the W-Beijing strain GC1237 competitively binds to TLR-2 to attenuate responses to canonical TLR-2 agonists, including mycobacterial lipomannans [25]. *M. tuberculosis* can also impair innate immune responses to cell envelope components by enzymatic methods. For instance, an *M. tuberculosis* serine hydrolase, Hip1, cleaved multimeric, cell wall-associated GroEL2 to a secreted monomeric form, which attenuated macrophage and DC responses [26, 27]. In addition,

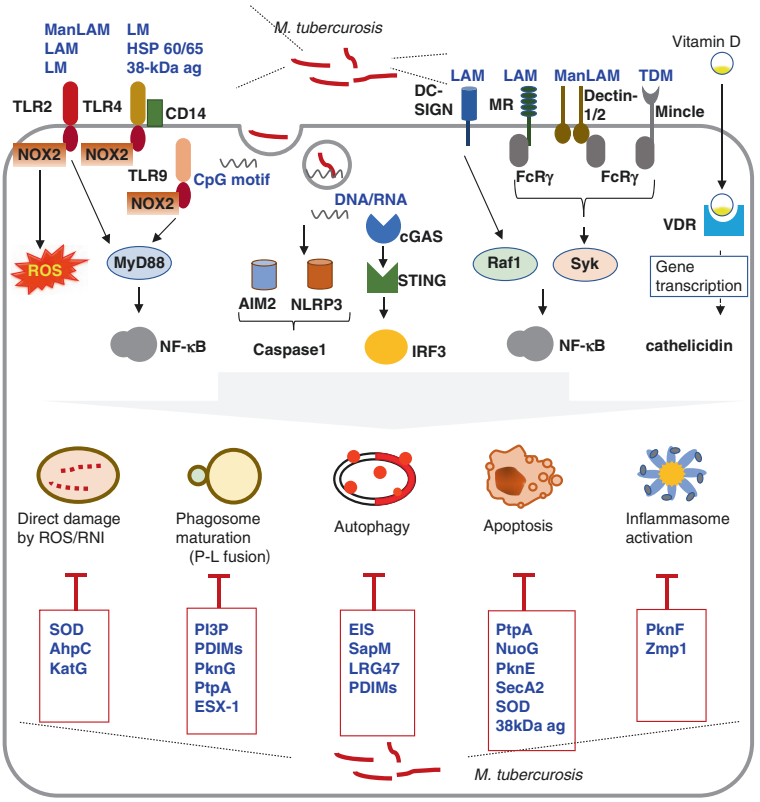

Fig. 2.2 Recognition of *M. tuberculosis* by pattern recognition receptors and evasion of host immune responses by *M. tuberculosis*. Innate immune cells, such as macrophages and dendritic cells, express various pattern recognition receptors (PRRs) to recognize pathogen-associated molecular patterns (PAMPs). Lipoglycans, the cell wall component of mycobacteria, including lipomannan (LM), lipoarabinomannan (LAM), and mannosylated lipoarabinomannan (ManLAM) are recognized by toll-like receptors (TLR)-2, and C-type lectins (e.g., Mannose receptors (MR), DC-SIGN, Dectin-1/2). Trehalose-6,6′-dimycolate (TDM), which contains long chain fatty acid mycolic acid, is recognized by macrophage-inducible C-type lectin (Mincle). These interactions activate NF-κB via intracellular signaling pathways. Heat shock protein (HSP) 60/65 and 38-kDa antigen (ag) of *M. tuberculosis* are recognized by TLR-4, and CpG motif of mycobacterial DNA is recognized by TLR-9. Besides activation of NF-κB, TLR activation induces the generation of reactive oxygen intermediates (ROS) by activating nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX)-2. *M. tuberculosis* DNA or bacterial second messengers can be recognized by cytosolic PRRs, such as cGAS/STING, Nod-like receptors (e.g., NLRP3), and absent in melanoma 2 (AIM2)-like receptors. Recognition of mycobacterial PAMPs by PRRs in innate immune cells cause several cellular events, including phagosome maturation, autophagy, apoptosis, inflammasome activation, and oxidative/nitrosative stresses, to eliminate bacteria. The vitamin D pathway enhances pathogen sensing, phagosomal maturation, and IFN-γ induction by transactivating cathelicidin gene. Several mycobacterial components interfere with these cellular functions to evade or allow adaptation to intracellular immune pressure. Syk; spleen tyrosine kinase, RAF; proto-oncogene serine/threonine-protein kinase, SOD; Superoxide Dismutase, AhpC; alkyl hydroperoxide reductases, KatG; catalase-peroxidase, PI3P; phosphatidylinositol 3-phosphate, PDIMs; phthiocerol dimycocerosates, Pkn; serine/threonine kinases, PtpA; protein tyrosine phosphatase A, ESX-1; early secreted antigenic target 6 kDa (ESAT-6) secretion system-1, EIS; the enhanced intracellular survival, SapM; secreted acid phosphatase, LRG47; IFN-γ inducible p47 GTPase, nuoG; NADH-quinone oxidoreductase subunit G, SecA2; protein translocase subunit SecA2, Zmp1; zinc metalloprotease 1

M. tuberculosis mutants lacking Hip1 or a putative mycobacterial metalloprotease, zmp1, displayed enhanced inflammasome activation [27, 28], suggesting that *M. tuberculosis* possesses multiple strategies for dampening activation of the inflammasome.

Thus, in addition to host receptors that mediate the recognition of *M. tuberculosis*, innate immune responses to infection likely depend on the strain of *M. tuberculosis,* the presence of cell wall components that competitively inhibit the activation of PRRs, and the presence of *M. tuberculosis* enzymes that modify the immunogenicity of cell envelope components.

2.2 Phagosomal Defense in Macrophages

Macrophages are the first immune cells to encounter *M. tuberculosis* during infection and are also the primary niche for mycobacterial replication. The recognition of *M. tuberculosis* by macrophages leads to the phagocytosis and sequestration of the bacteria in phagosomes, which typically kill pathogens via fusion with lysosomes and the subsequent acidification of the pathogen-containing phagolysosome. However, *M. tuberculosis* can survive and replicate in the phagosome by inhibiting phagosomal maturation and phagolysosomal generation through a variety of mechanisms [7, 29]. The transcriptional profiling of intraphagosomal bacteria indicated that *M. tuberculosis* expresses several stress-adaptive genes that counter the nitrosative, oxidative, hypoxic, and nutrient-poor phagosomal environment [30]. A genome-wide transposon site hybridization screening also suggested that *M. tuberculosis* constitutively expresses genes required for its survival [31]. Thus, *M. tuberculosis* can adapt its lifestyle via various strategies to survive inside macrophages. *M. tuberculosis* glycolipids prevent phagolysosome biosynthesis by inhibiting the accumulation of phosphatidylinositol 3-phosphate (PI3P) on phagosomal membranes [32]. *M. tuberculosis* also secretes phosphatases (SapM and PtpA) and serine/threonine kinases (PknG) that are proposed to interfere with phagosomal maturation [33–35]. One study has also reported that *M. tuberculosis* lipids, in particular PDIMs, contribute to phagosomal escape of the bacteria and host cell death [36]. An *M. tuberculosis* secretion system, ESX-1, mediates the disruption of phagosomal integrity and prevents phagosome maturation. The promotion of aberrant phagosomal integrity and mycobacterial replication by ESX-1 is countered by IFN-γ-inducible Rab20-mediated phagosomal maturation [37]. The ESX-1-mediated phagosomal escape of bacteria is mediated by a 6-kDa early secretory antigenic target (ESAT)-6, which enhances mycobacterial cytosolic translocation within host macrophages by rupturing their phagosomal membranes [38–40]. In addition to ESAT-6, recent evidence has proposed a contact-dependent, ESAT-6-independent mechanism for ESX-1-mediated phagosomal permeabilization [41].

Mycobacterial entry into macrophages through different receptors can lead to the activation of distinct signaling pathways that inhibit or promote bacterial replication. For example, *M. tuberculosis* uptake by complement receptor 3 (CR3)

depended on host cholesterol, which mediates the recruitment of the leukocyte-specific protein coronin-1 to phagosomes and the subsequent inhibition of phagolysosome formation through the activation of host calcineurin [42, 43]. The TLR-2 recognition of mycobacterial ManLAM activates NF-κB and nitric oxide synthase (NOS) 2 gene transcription, which leads to NO production [44]. The antimycobacterial effects of NO have been reported in vitro and in vivo. Reactive nitrogen intermediates (RNI), formed by interactions between L-arginine and NO, are toxic to mycobacteria [45–47] and infection can be exacerbated by the inhibition of NOS [48, 49, 50, 51]. Furthermore, mice with disrupted NOS2 alleles had exacerbated *M. tuberculosis* infection [50, 52]. However, *M. tuberculosis* has several strategies to cope with damage caused by RNI and reactive oxygen intermediates (ROS): *M. tuberculosis* KatG, a catalase-peroxidase, can inactivate phagosomal ROS [53] and the *M. tuberculosis* proteasome can mediate resistance to nitrosative stresses [54]. TLR-2 signaling is essential for the induction of protective immune responses to mycobacteria, including the induction of TNF-α, in macrophages [55]. The TLR-mediated recognition of *M. tuberculosis* was reported to synergize with the vitamin D pathway to induce the antimicrobial peptide, cathelicidin, in human macrophages [56, 57]. The biologically active vitamin D metabolite, calcitriol, induces hCAP-18, a gene encoding the pro-form of cathelicidin, following TLR ligation on macrophages [56, 57]. In addition to direct antimicrobial activity, cathelicidin exerts antimicrobial functions by inducing the host autophagy genes Beclin-1 and Atg5 [58]. The vitamin D pathway also synergizes with IFN-γ secreted by T-cells to induce IL-15 autocrine signaling, which promotes autophagy and phagosomal maturation in *M. tuberculosis*-infected human macrophages [59].

Autophagy is a process whereby cytoplasmic constituents are degraded or recycled. The role of autophagy in antimycobacterial immunity in macrophages has been extensively characterized. Initial studies using *M. bovis* suggested that autophagy promotes phagosomal maturation to enhance bacterial killing [60]. Moreover, LRG-47—an IFN-γ inducible p47 GTPase that is critical for phagosomal maturation and control of *M. tuberculosis* [61]—is also involved in the induction of autophagy in *M. bovis*-infected macrophages [62]. Autophagy-related genes were revealed to be involved in regulating the intracellular bacterial load of *M. tuberculosis* in a genome-wide siRNA screening of infected human macrophage-like THP-1 cells [63]. Accumulating evidence indicates that autophagy is integrated into the host response to *M. tuberculosis* infection by synergizing with pathogen sensing, phagosomal maturation, and IFN-γ inducible pathways to mediate antimycobacterial immunity [9, 12, 64, 65]. However, *M. tuberculosis* has several strategies to inhibit autophagy and reprogram host lipid metabolism to enable intracellular survival and persistence in the host [66, 67].

Taken together, it is clear that macrophage recognition and phagocytosis of *M. tuberculosis* lead to a dynamic competition between antimycobacterial defense systems and *M. tuberculosis* immune evasion. Macrophage defense systems include antimicrobial peptides, nitrosative stresses, phagolysosomal fusion, and autophagy, which might operate independently of, or subsequent to, IFN-γ signaling. However, *M. tuberculosis* can subvert macrophage defense systems at the level of the bacterial

cell wall components that limit phagosomal maturation, and bacterial genes that combat or allow adaptation to intracellular immune pressure. The relationship between *M. tuberculosis* and macrophages is shown in Fig. 2.2.

3 Initiation of Adaptive Immunity to *M. Tuberculosis* by DCs

An important function of innate immunity during *M. tuberculosis* infection is the priming of adaptive immune responses. DCs are professional antigen-presenting cells that initiate adaptive immunity by presenting *M. tuberculosis* antigens in the context of major histocompatibility complex (MHC), costimulatory molecules, and cytokines. pCD11c-diptheria toxin receptor/GFP transgenic mice, in which DCs are depleted, exhibit impaired bacterial control and delayed initiation of adaptive immunity after *M. tuberculosis* infection, illustrating the importance of DCs in mobilizing adaptive immune responses that can control bacterial replication [68]. Upon *M. tuberculosis* infection, DCs mature and migrate to the lung draining lymph nodes to initiate antigen-specific T-cell responses, which depend on the chemokine receptor CCR7 and its corresponding chemokines CCL19 and CCL21 [69–71]. IL-12—a cytokine secreted by myeloid cells and involved in the induction of IFN-γ responses—is required for DC migration during *M. tuberculosis* infection [72]. The priming of adaptive immune responses requires the transport of live bacteria to the lung draining lymph nodes [69, 73], but antigen-specific T-cells can be primed by infected migratory DCs or uninfected lymph node resident DCs. Effective interactions between DCs and T-cells depend on the appropriate function of antigen presentation machinery, including the expression of MHC, costimulatory molecules, and cytokines following *M. tuberculosis* infection. Therefore, DCs are critical factors that initiate adaptive immune responses to *M. tuberculosis* and determine the outcome of infection. Interventions or therapies that improve DC functions may provide benefit by augmenting crosstalk between DCs and antigen-specific T-cells.

4 The Role of Adaptive Immunity against *M. Tuberculosis*

Protective immunity to *M. tuberculosis* and the control of bacterial replication require adaptive immune responses. HIV patients and mice lacking MHC class II or T-cells are extremely susceptible to mycobacterial infections, indicating the importance of adaptive immune responses for controlling mycobacterial infection. Cytokine secretion and the direct antimicrobial action of antigen-specific T-cells are key features of the adaptive immune response against *M. tuberculosis* infection. Here, we discuss the importance of the IFN-γ/IL-12 axis, which involves Th1 cells and macrophages, and how CD8 T-cells contribute to immunity against *M. tuberculosis*.

4.1 The IFN-γ/IL-12 Axis and TB Immunity

As mentioned above, *M. tuberculosis* has a variety of methods to counteract host bactericidal activity within macrophages. Therefore, it is difficult for macrophages alone to sterilize TB. T cells activated by acquired immunity activate macrophages, which effectively sterilize TB. This important mechanism of TB immunity is termed the IFN-γ/IL-12 axis.

The production of IFN-γ by Th1 cells, CD8 T-cells, and other lymphocytes is considered essential for protection against mycobacterial infections. In human immunogenetics studies, Mendelian susceptibility to mycobacterial disease (MSMD) shows a spectrum of genetic mutations in five autosomal genes (*IFNGR1, IFNGR2, STAT1, IL12B,* and *IL12RB*) and an X-linked MSMD, which confers susceptibility to avirulent environmental mycobacteria and BCG [74]. Deficiencies related to IFN-γ signaling in young patients with mutations in IFNGR1 and IFNGR2 confer fatal susceptibility to mycobacterial infections [75–78]. STAT1 is an intracellular molecule important for IFN-γ signaling and individuals with heterozygous germline STAT1 mutations lose gamma-interferon activating factor (GAF) expression [79]. GAF is an important transcription factor that facilitates IFN-γ-induced gene expression. Individuals with heterozygous STAT1 mutations have an impaired nuclear accumulation of GAF and suffer from recurrent mycobacterial infections [79].

Gene mutations affecting IL-12 expression levels and signaling also confer susceptibility to mycobacterial infections. Two mutations in the leucine zipper domain of NEMO, which is an intracellular protein involved in NF-κB activation, impair CD40-mediated IL-12 production in monocytes and DCs [80], leading to recurrent mycobacterial infections. Similarly, gene defects that impair IL-12p40 lead to decreased IFN-γ levels and confer susceptibility to mycobacterial infections [81–84]. Mutations in IL12RB are the most frequent genetic factors associated with MSMD, but recurrent mycobacterial susceptibility in individuals with IL12RB mutations can be mitigated with BCG vaccination or primary BCG disease [81, 82, 85–88], suggesting that IL-12/IL-23 signaling may not be completely required for secondary immunity.

Animal models of TB have also demonstrated the key role of IFN-γ in immunity against *M. tuberculosis* infection. Mice deficient in IFN-γ succumbed to low-dose *M. tuberculosis* infection [89, 90]. Correspondingly, mice lacking IL-12 were also unable to control *M. tuberculosis* infection [91, 92]. The antimycobacterial effects of IFN-γ in mouse models are broadly related to the induction of the antimicrobial peptides, iNOS, NRAMP1, and cytokines that activate infected macrophages to restrict intracellular bacterial replication [93].

T-bet, which is a member of the T-box family of transcription factors encoded by Tbx21, is the master transcriptional regulator for lineage commitment to the Th1 subset [94, 95]. Mice lacking T-bet are susceptible to virulent *M. tuberculosis* infection [96]. The susceptibility of T-bet-deficient mice is associated with increased

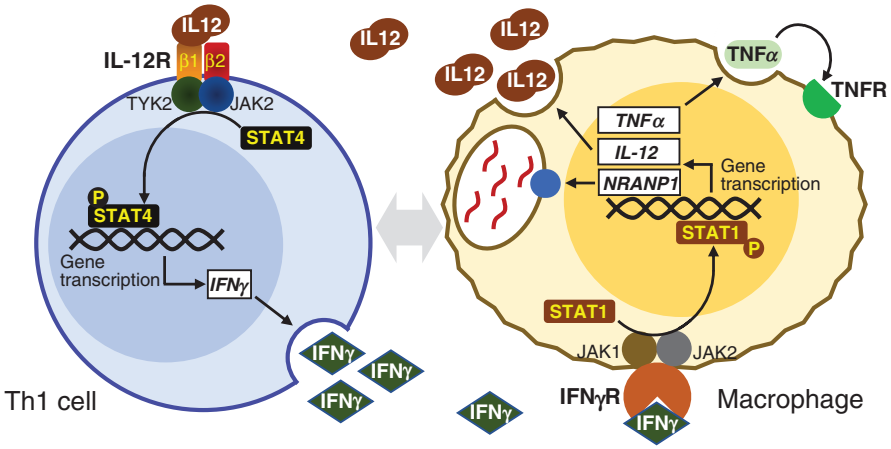

Fig. 2.3 Antimycobacterial immunity induced by IFN-γ and IL-12. Mycobacterial immunity requires IFN-γ and IL-12 between Th1 cells and macrophages. IL-12, produced by infected macrophages, differentiates naïve T cells to Th1 cells and activates them to produce IFN-γ, which binds to its cognate receptor (IFNγR) expressed on macrophages, leading to signal transducer and activator of transcription 1 (STAT1) phosphorylation, dimerization, nuclear translocation, and the transcription of several antimycobacterial genes such as natural resistance-associated macrophage protein 1 (NRAMP1). Macrophages also produce TNF-α in an autocrine manner, which activates themselves, thereby contributing to the sterilization of TB. This control mechanism of mycobacterial infection is termed the IFN-γ/IL-12 axis

systemic bacterial burden, diminished IFN-γ production, and the marked accumulation of eosinophilic macrophages and multinucleated giant cells in the lung. T-bet-deficient mice exhibited a selective increase in IL-10 production. This indicated that T-bet has a central role in controlling *M. tuberculosis* disease progression, in part through the regulation of IFN-γ and IL-10 [96]. IL-10 deficient mice are less susceptible to *M. tuberculosis* infection because of an enhanced Th1 response [97], suggesting that IL-10 limits Th1 immunity during *M. tuberculosis* infection. The role of the IFN-γ/IL-12 axis in antimycobacterial immunity is shown in Fig. 2.3.

4.2 The Role of CD8 T-Cells in M. Tuberculosis *Infection*

Mice with a gene deletion of β2 microglobulin, which abrogates MHC class I antigen presentation, or mice depleted of CD8 T-cells, live longer than mice with corresponding disruptions to the MHC class II pathway or CD4 T-cell responses following *M. tuberculosis* infection [98]. Regardless, CD8 T-cells contribute significantly to immunity against *M. tuberculosis* infection. Mice lacking transporter associated with antigen processing 1 (TAP-1) antigen presentation molecules exhibited aberrant CD8 T-cell responses and succumbed more rapidly following *M.*

tuberculosis infection compared with wild-type controls [99, 100]. The depletion of CD8 T-cells in Rhesus macaques compromised protective immunity from BCG vaccination or chemotherapeutic interventions [101], suggesting that CD8 T-cells are an important component of recall responses to *M. tuberculosis* infection. A mouse model of *M. tuberculosis* infection also revealed the importance of CD8 T-cell responses in preventing reactivation [102]. The importance of CD8 T-cells during *M. tuberculosis* infection is related to their secretion of cytokines and cytolytic effector molecules that can limit bacterial replication. In addition to IFN-γ and TNF-α, CD8 T-cells secrete perforin, which lyses *M. tuberculosis*-infected macrophages [103]. CD8 T-cells also release granulysin, which directly kills intracellular *M. tuberculosis* [104, 105]. The use of anti-TNF-α therapy in patients with rheumatoid arthritis depletes a subset of effector memory CD8 T-cells that secrete granulysin and express cell surface TNF [106], which may partially explain the increased progression from LTBI to active TB in patients undergoing anti-TNF-α therapy. Human CD8 T-cells respond to epitopes in CFP10 [107], ESAT-6 [108, 109], and the Ag85 complex [110, 111]. A variety of human CD8 T-cell clones tested against a panel of synthetic peptides derived from immunodominant *M. tuberculosis* antigens revealed that CD8 T-cell responses are concentrated toward a limited set of epitopes and are generally restricted by the HLA-B allele [112, 113].

Taken together, CD8 T-cells are a critical component of adaptive immunity to *M. tuberculosis* infection and have an important role in different disease contexts by limiting reactivation during latency and by directly participating in antimicrobial functions during active infections.

5 LTBI and its Reactivation

Approximately 5%–10% of individuals infected with *M. tuberculosis* develop disease during the first 2–5 years after infection [114]. For other individuals, the innate immune response will either fully eliminate the infection without leaving a trace of immunological response (resistance to TB infection) [115] or lead to a state of persistent immune response to *M. tuberculosis* antigens without clinical evidence of active disease [114, 115]. This last outcome is the basis for considering that one-fourth of the world population is infected with *M. tuberculosis* [116]. These are individuals who have persistent LTBI immunoreactivity, even when bacterial clearance has been achieved, thus becoming a potential reservoir for active TB.

As mentioned above, previous reports have underscored the importance of adaptive immunity, particularly CD4 T cells, and their production of TNF-α and IFN-γ to control TB. Therefore, these host immunities are strongly involved in the establishment of LTBI. In addition, many studies have highlighted the importance of various adaptive mechanisms in promoting the long-term survival of *M. tuberculosis* in host tissues, including the stringent response and a switch to the utilization of fatty acids as a source of carbon and energy through the glyoxylate shunt [117–119].

The goal of LTBI treatment is to prevent reactivation, and this is especially recommended for persons who are at increased risk for progression from LTBI to disease [120]. Persons at increased risk of reactivation of LTBI include those with HIV/AIDS, those receiving immunosuppressive treatment, including cancer chemotherapy, systemic steroids, and anti-TNF agents, and those with chronic systemic diseases, such as end-stage renal disease, rheumatic disorders, and diabetes mellitus [121]. Attesting to the importance of CD4 T cells in host defenses during LTBI, the risk of TB reactivation increases dramatically in patients with HIV coinfection, from 5%–10% per lifetime in immunocompetent hosts to as high as 10% annually in coinfected patients [122], and the risk of TB reactivation rises as the CD4 cell count declines [123, 124]. Recently, the role of the inhibition of TNF-α signaling and PD-1 pathways on the pathogenesis of LTBI has been reported.

5.1 TNF-α Inhibitors and Mycobacterial Infections

TNF-α is a potent inflammatory cytokine that has an important role in immunity to numerous bacterial infections, including *M. tuberculosis* [125, 126]. TNF-α is critical for granuloma formation and might be required for granuloma maintenance [127]. Adhesion molecules may also be regulated by TNF-α, which is critical for granuloma formation and the control of infection [128, 129].

Infliximab, adalimumab, certolizumab pegol, and etanercept are anti-TNF-α agents used to treat a range of inflammatory/autoimmune diseases, such as rheumatoid arthritis. The use of these drugs has been linked to the reactivation of TB. In addition to blocking TNF-α-mediated immune responses, some anti-TNF-α drugs interfere with innate immune responses, such as phagolysosomal maturation and monocyte apoptosis, as well as cell-mediated responses, including IFN-γ secretion by memory T cells, the complement-mediated lysis of *M. tuberculosis* reactive CD8 positive T cells, and increased regulatory T cell activity [130–135].

All TNF-α inhibitors increase the risk of TB [136–138], although the risk is greater for the anti-TNF-α monoclonal antibodies infliximab and adalimumab than for the soluble receptor fusion protein etanercept [136–138]. The risk associated with the newer monoclonal agents certolizumab and golimumab has not been established in population-based studies or compared with other TNF-α inhibitors. However, TB cases have been reported after the use of these agents, and their risk is presumed to be similar to the other monoclonal anti-TNF agents [139, 140]. Many TB cases associated with TNF-α inhibitors likely represent the reactivation of LTBI; therefore indicating the rationale for screening for LTBI before initiating therapy. In areas of low TB prevalence, a minority of TNF-α inhibitor-associated cases represent newly acquired infection. A schema of the role of TNF-α inhibitors and immunity to TB is shown in Fig. 2.4.

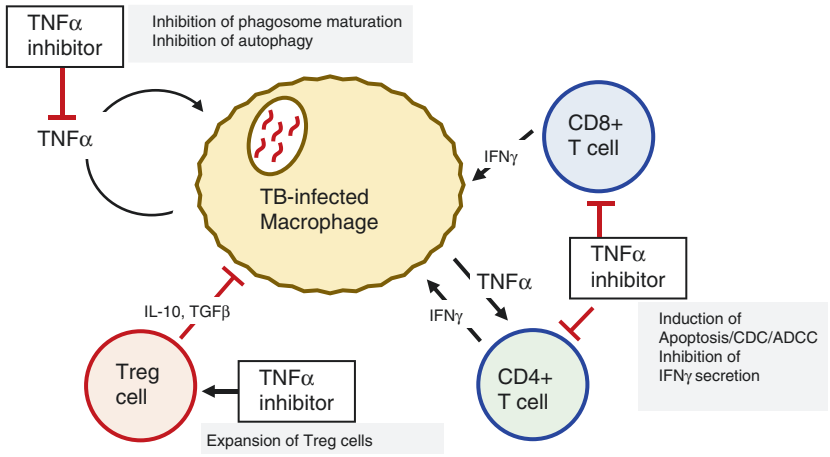

Fig. 2.4 TNF-α inhibitors and mycobacterial infections. TNF-α is induced in infected macrophages and enhances P-L fusion and autophagy so that phagosome maturation is inhibited by TNF-α inhibitors. Some TNF-α inhibitors induce the killing of T cells by apoptosis, complement-dependent cytotoxicity, and antibody-dependent cell-mediated cytotoxicity. TNF-α inhibitors may reduce IFN-γ responses by an increase in IL-10 production. Thus, TNF-α inhibitors increase the risk of TB reactivation when they are used to treat LTBI subjects

5.2 Role of the PD-1 Pathway in TB

PD-1 was identified as a surface antigen on apoptotic T cells [141]. Subsequent analysis showed that PD-1 is expressed on activated T cells and B cells [142] and is homeostatically expressed on dysfunctional CD4-positive T cells [143]. PD-1 functions as a receptor and binds to two specific ligands, PD-L1 and PD-L2: PD-L1 is expressed on a variety of cells, whereas PD-L2 is expressed only on activated DCs and macrophages [144]. Moreover, it was reported that the expression of PD-L1 on antigen-presenting cells is enhanced by stimulation with IFN-γ or TLRs [145]. Functional analysis of the PD-1 pathway revealed that the binding of PD-1 to PD-L1 or PD-L2 transmits inhibitory signals to T cells when antigen-presenting cells present antigens to T cells.

This inhibitory signal is important for maintaining immune tolerance to suppress autoimmune responses in peripheral tissues [146]. However, PD-1-mediated inhibitory signaling inhibited pathogen elimination in chronic viral infections, such as lymphocytic choriomeningitis virus, HIV, and hepatitis B virus [147–149]. These results indicate that the PD-1 signaling pathway has an important role in regulating T cell activation and suppressing tissue injury, but may also make it difficult to eliminate chronically infected pathogens.

PD-L1 is expressed on normal immune cells as well as cancer cells. T cell functions are reduced and antitumor immunity is suppressed when PD-L1 on cancer cells binds to PD-1 on T cells. Immune checkpoint inhibition (ICI) therapy, which has recently attracted increased attention for cancer treatment, is a new concept of

cancer treatment in which the immunosuppressive mechanism is released by administering antibodies that bind to PD-1 or PD-L1. As a result, ICI treatments activate T cell responses and enhance antitumor immunity. In this situation, Th1 immunity is activated and it was predicted that TB infection would also be improved. However, TB infection was reported to be reactivated when ICI treatment was administered to cancer patients [150, 151]. PD-1 and PD-L1 are expressed in human TB granulomas, suggesting a regulatory role at the site of disease [151]. Recently, TB-specific immune responses were analyzed in a case of immune checkpoint-associated TB [152]. CD4 T cells that produce TB-specific IFN-γ were increased in the peripheral blood and granuloma formation with multinucleated giant cells, and central necrosis was observed in the lung after treatment with a PD-1 inhibitor. However, there was no change in interleukin-17 (IL-17)-producing CD4 and CD8 T cells. These findings indicate that PD-1 has an inhibitory function in the development of TB and that the use of PD-1 inhibitors in cancer therapy may exacerbate the pathogenesis of TB.

According to a recent study [153] that performed a literature review and analyzed 562 patients with advanced lung cancer who received anti-PD-1/PD-L1 immunotherapy, ICI therapy was relatively safe for cancer patients complicated with previously treated latent TB infection, and the efficacy of ICI therapy in this specified population was not inferior to that in lung cancer patients without TB infection. However, it is widely accepted that the blockade of PD-1/PD-L1 is associated with the development or reactivation of TB [153].

Studies using animal models have shown that CD4 T cells, which normally act defensively against infection, overproduce IFN-γ resulting in lethal lung tissue destruction when PD-1-deficient mice are infected with *M. tuberculosis* in the lungs [154, 155]. Interestingly, previous studies also have shown that stimulating *M. tuberculosis*-infected macrophages with high concentrations of IFN-γ led to the death of macrophages instead of the expected killing of the bacteria [156]. However, a recent study using a microsphere model reported that the inhibition of PD-1 signaling increased *M. tuberculosis* growth and augmented cytokine secretion [157]. TNF-α accelerated *M. tuberculosis* growth and TNF-α neutralization reversed the augmented *M. tuberculosis* growth caused by anti-PD-1 treatment. They concluded that PD-1 regulated the immune response in TB, and that the inhibition of PD-1 accelerated *M. tuberculosis* growth via excessive TNF-α secretion [157]. Rhesus macaques treated with anti-PD-1 antibody developed worse TB disease and higher granuloma bacterial loads compared with controls [158]. PD-1 blockade increased the number and functionality of granuloma *M. tuberculosis*-specific CD8 T cells. In contrast, *M. tuberculosis*-specific CD4 T cells in anti-PD-1-treated macaques were not increased in number or function in granulomas; however, they exhibited reduced intralesional trafficking in live imaging studies. They also showed that caspase 1 activation was required for the exacerbation of *M. tuberculosis* infection after PD-1 blockade and that the levels of IFN-γ, TNF-α, and IL-18 were elevated in granulomas of anti-PD-1 treated macaques. They discussed that Th1 cell functions should be appropriately balanced for the optimal control of TB infection [158].

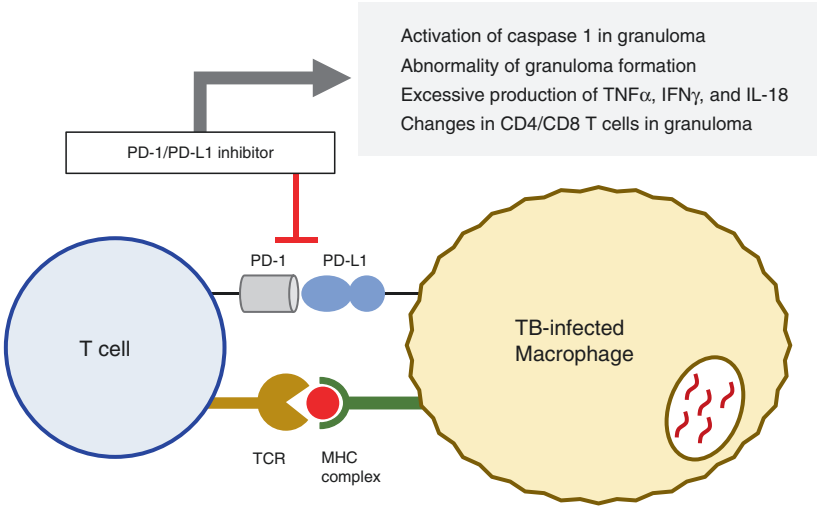

Fig. 2.5 Role of PD-1 pathway suppression in TB. Blockade of the PD-1 pathway potentiates adaptive immune responses in infectious diseases. Inhibition of the PD-1 signaling pathway, initiated upon interactions between PD-1 and PD-L1/2, leads to the activation of exhausted antigen-specific T-cells in cancer. The same is envisaged for pathogen-specific T-cells in infectious diseases as seen in chronic viral infections. Activation of these antigen-specific T-cells promotes their ability to produce proinflammatory cytokines, including IFN-γ and TNF-α, as well as cytolytic molecules such as perforin, granzyme B, and granulysin, which can lyse and kill transformed cells to control the spread and burden of disease. PD-1 pathway suppression also activates caspase 1 and enhances the production of TNF-α, IFN-γ, and IL-18 in granulomas. Furthermore, an increase in the number of CD8-positive cells and a decrease in the invasive ability of CD4-positive cells into granulomas occur, resulting in a marked increase in the number of *M. tuberculosis* bacteria in the granuloma

The mechanism of TB development or exacerbation by PD-1 inhibition is not fully understood. Putative mechanisms derived from these reports are shown in Fig. 2.5. Detailed pathological mechanisms related to PD-1 inhibition and TB exacerbation need to be elucidated in the future.

6 Conclusion

Answer to Clinical Questions:

1. How does the immune system affect the development of pulmonary TB?

   When *M. tuberculosis* invades the body, innate immunity is activated. If the number of bacilli is low, innate immunity alone can eliminate TB bacilli. However, if the number of bacilli is high, acquired immunity is induced because *M. tuberculosis* counteracts the bactericidal activity in macrophages. In acquired immunity, the interaction between infected macrophages and T-cells, especially

Th1 cells, is essential for the effective elimination of mycobacteria. IL-12, produced by infected macrophages, differentiates naïve T cells to Th1 cells and activates them to produce IFN-γ, which induces mycobactericidal activity in infected macrophages. Macrophages also produce TNF-α in an autocrine manner, which activates themselves, thereby contributing to the sterilization of TB. This control mechanism of mycobacterial infection is termed the IFN-γ/IL-12 axis. The disruption or suppression of this axis causes TB infection and exacerbates TB infection.

2. How does an understanding of TB immunology help clinicians manage their patients with pulmonary TB?

   Recently, there has been an increase in the use of drugs that affect T cell immunity, such as anti-TNF-α agents and ICIs. As a result, there are concerns about the impact of these drugs on TB immunity. The suppression of the IFN-γ/IL-12 axis and TNF-α, which are key components of TB immunity, leads to the development and progression of TB. Although the inhibition of PD-1/PD-L1 pathways was expected to activate Th1 immunity and to act as a protective effect against TB infection, in contrast to expectations, inhibition of PD-1/PD-L1 pathways led to the development and progression of TB. These factors indicate that T cell immunity needs to be appropriately balanced for the optimal control of TB infection. Effects on the susceptibility to TB must be considered when a new drug that affects T cell immunity is developed.

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References

1. Abel L, El-Baghdadi J, Bousfiha AA, Casanova JL, Schurr E. Human genetics of tuberculosis: a long and winding road. Philos Trans R Soc Lond Ser B Biol Sci. 2014;369:20130428. https://doi.org/10.1098/rstb.2013.0428.
2. Yamamura Y. The pathogenesis of tuberculous cavities. Bibl Tuberc. 1958;13:13–37.
3. San KE, Muhamad M. Pulmonary tuberculosis in HIV infection: the relationship of the radiographic appearance to CD4 T-lymphocytes count. Malays J Med Sci. 2001;8:34–40.
4. Gupta N, Kumar R, Agrawal B. New players in immunity to tuberculosis: the host microbiome, lung epithelium, and innate immune cells. Front Immunol. 2018;9:709. https://doi.org/10.3389/fimmu.2018.00709.
5. Thoma-Uszynski S, Stenger S, Takeuchi O, Ochoa MT, Engele M, Sieling PA, Barnes PF, Rollinghoff M, Bolcskei PL, Wagner M, et al. Induction of direct antimicrobial activity through mammalian toll-like receptors. Science. 2001;291:1544–7. https://doi.org/10.1126/science.291.5508.1544.
6. Jo EK, Yang CS, Choi CH, Harding CV. Intracellular signalling cascades regulating innate immune responses to mycobacteria: branching out from toll-like receptors. Cell Microbiol. 2007;9:1087–98. https://doi.org/10.1111/j.1462-5822.2007.00914.x.
7. Philips JA, Ernst JD. Tuberculosis pathogenesis and immunity. Annu Rev Pathol. 2012;7:353–84. https://doi.org/10.1146/annurev-pathol-011811-132458.

8. Kleinnijenhuis J, Oosting M, Joosten LA, Netea MG, Van Crevel R. Innate immune recognition of mycobacterium tuberculosis. Clin Dev Immunol. 2011;2011:405310. https://doi.org/10.1155/2011/405310.
9. Watson RO, Manzanillo PS, Cox JS. Extracellular M. tuberculosis DNA targets bacteria for autophagy by activating the host DNA-sensing pathway. Cell. 2012;150:803–15. https://doi.org/10.1016/j.cell.2012.06.040.
10. Manzanillo PS, Shiloh MU, Portnoy DA, Cox JS. Mycobacterium tuberculosis activates the DNA-dependent cytosolic surveillance pathway within macrophages. Cell Host Microbe. 2012;11:469–80. https://doi.org/10.1016/j.chom.2012.03.007.
11. Dey B, Dey RJ, Cheung LS, Pokkali S, Guo H, Lee JH, Bishai WR. A bacterial cyclic dinucleotide activates the cytosolic surveillance pathway and mediates innate resistance to tuberculosis. Nat Med. 2015;21:401–6. https://doi.org/10.1038/nm.3813.
12. Collins AC, Cai H, Li T, Franco LH, Li XD, Nair VR, Scharn CR, Stamm CE, Levine B, Chen ZJ, et al. Cyclic GMP-AMP synthase is an innate immune DNA sensor for mycobacterium tuberculosis. Cell Host Microbe. 2015;17:820–8. https://doi.org/10.1016/j.chom.2015.05.005.
13. Watson RO, Bell SL, MacDuff DA, Kimmey JM, Diner EJ, Olivas J, Vance RE, Stallings CL, Virgin HW, Cox JS. The cytosolic sensor cGAS detects mycobacterium tuberculosis DNA to induce type I interferons and activate autophagy. Cell Host Microbe. 2015;17:811–9. https://doi.org/10.1016/j.chom.2015.05.004.
14. Fremond CM, Yeremeev V, Nicolle DM, Jacobs M, Quesniaux VF, Ryffel B. Fatal mycobacterium tuberculosis infection despite adaptive immune response in the absence of MyD88. J Clin Invest. 2004;114:1790–9. https://doi.org/10.1172/jci21027.
15. Scanga CA, Bafica A, Feng CG, Cheever AW, Hieny S, Sher A. MyD88-deficient mice display a profound loss in resistance to mycobacterium tuberculosis associated with partially impaired Th1 cytokine and nitric oxide synthase 2 expression. Infect Immun. 2004;72:2400–4. https://doi.org/10.1128/iai.72.4.2400-2404.2004.
16. Mayer-Barber KD, Barber DL, Shenderov K, White SD, Wilson MS, Cheever A, Kugler D, Hieny S, Caspar P, Núñez G, et al. Caspase-1 independent IL-1beta production is critical for host resistance to mycobacterium tuberculosis and does not require TLR signaling in vivo. J Immunol. 2010;184:3326–30. https://doi.org/10.4049/jimmunol.0904189.
17. Fremond CM, Togbe D, Doz E, Rose S, Vasseur V, Maillet I, Jacobs M, Ryffel B, Quesniaux VF. IL-1 receptor-mediated signal is an essential component of MyD88-dependent innate response to mycobacterium tuberculosis infection. J Immunol. 2007;179:1178–89. https://doi.org/10.4049/jimmunol.179.2.1178.
18. Shi S, Nathan C, Schnappinger D, Drenkow J, Fuortes M, Block E, Ding A, Gingeras TR, Schoolnik G, Akira S, et al. MyD88 primes macrophages for full-scale activation by interferon-gamma yet mediates few responses to mycobacterium tuberculosis. J Exp Med. 2003;198:987–97. https://doi.org/10.1084/jem.20030603.
19. Velez DR, Wejse C, Stryjewski ME, Abbate E, Hulme WF, Myers JL, Estevan R, Patillo SG, Olesen R, Tacconelli A, et al. Variants in toll-like receptors 2 and 9 influence susceptibility to pulmonary tuberculosis in Caucasians, African-Americans, and west Africans. Hum Genet. 2010;127:65–73. https://doi.org/10.1007/s00439-009-0741-7.
20. Ma X, Liu Y, Gowen BB, Graviss EA, Clark AG, Musser JM. Full-exon resequencing reveals toll-like receptor variants contribute to human susceptibility to tuberculosis disease. PLoS One. 2007;2:e1318. https://doi.org/10.1371/journal.pone.0001318.
21. Davila S, Hibberd ML, Hari Dass R, Wong HE, Sahiratmadja E, Bonnard C, Alisjahbana B, Szeszko JS, Balabanova Y, Drobniewski F, et al. Genetic association and expression studies indicate a role of toll-like receptor 8 in pulmonary tuberculosis. PLoS Genet. 2008;4:e1000218. https://doi.org/10.1371/journal.pgen.1000218.
22. Khor CC, Chapman SJ, Vannberg FO, Dunne A, Murphy C, Ling EY, Frodsham AJ, Walley AJ, Kyrieleis O, Khan A, et al. A mal functional variant is associated with protection

against invasive pneumococcal disease, bacteremia, malaria and tuberculosis. Nat Genet. 2007;39:523–8. https://doi.org/10.1038/ng1976.
23. Reed MB, Domenech P, Manca C, Su H, Barczak AK, Kreiswirth BN, Kaplan G, Barry CE 3rd. A glycolipid of hypervirulent tuberculosis strains that inhibits the innate immune response. Nature. 2004;431:84–7. https://doi.org/10.1038/nature02837.
24. Sinsimer D, Huet G, Manca C, Tsenova L, Koo MS, Kurepina N, Kana B, Mathema B, Marras SA, Kreiswirth BN, et al. The phenolic glycolipid of mycobacterium tuberculosis differentially modulates the early host cytokine response but does not in itself confer hypervirulence. Infect Immun. 2008;76:3027–36. https://doi.org/10.1128/iai.01663-07.
25. Blanc L, Gilleron M, Prandi J, Song OR, Jang MS, Gicquel B, Drocourt D, Neyrolles O, Brodin P, Tiraby G, et al. Mycobacterium tuberculosis inhibits human innate immune responses via the production of TLR2 antagonist glycolipids. Proc Natl Acad Sci U S A. 2017;114:11205–10. https://doi.org/10.1073/pnas.1707840114.
26. Rengarajan J, Murphy E, Park A, Krone CL, Hett EC, Bloom BR, Glimcher LH, Rubin EJ. Mycobacterium tuberculosis Rv2224c modulates innate immune responses. Proc Natl Acad Sci U S A. 2008;105:264–9. https://doi.org/10.1073/pnas.0710601105.
27. Madan-Lala R, Peixoto KV, Re F, Rengarajan J. Mycobacterium tuberculosis Hip1 dampens macrophage proinflammatory responses by limiting toll-like receptor 2 activation. Infect Immun. 2011;79:4828–38. https://doi.org/10.1128/iai.05574-11.
28. Master SS, Rampini SK, Davis AS, Keller C, Ehlers S, Springer B, Timmins GS, Sander P, Deretic V. Mycobacterium tuberculosis prevents inflammasome activation. Cell Host Microbe. 2008;3:224–32. https://doi.org/10.1016/j.chom.2008.03.003.
29. Ehrt S, Schnappinger D. Mycobacterial survival strategies in the phagosome: defence against host stresses. Cell Microbiol. 2009;11:1170–8. https://doi.org/10.1111/j.1462-5822.2009.01335.x.
30. Schnappinger D, Ehrt S, Voskuil MI, Liu Y, Mangan JA, Monahan IM, Dolganov G, Efron B, Butcher PD, Nathan C, et al. Transcriptional adaptation of mycobacterium tuberculosis within macrophages: insights into the Phagosomal environment. J Exp Med. 2003;198:693–704. https://doi.org/10.1084/jem.20030846.
31. Rengarajan J, Bloom BR, Rubin EJ. Genome-wide requirements for mycobacterium tuberculosis adaptation and survival in macrophages. Proc Natl Acad Sci U S A. 2005;102:8327–32. https://doi.org/10.1073/pnas.0503272102.
32. Fratti RA, Chua J, Vergne I, Deretic V. Mycobacterium tuberculosis glycosylated phosphatidylinositol causes phagosome maturation arrest. Proc Natl Acad Sci U S A. 2003;100:5437–42. https://doi.org/10.1073/pnas.0737613100.
33. Saleh MT, Belisle JT. Secretion of an acid phosphatase (SapM) by mycobacterium tuberculosis that is similar to eukaryotic acid phosphatases. J Bacteriol. 2000;182:6850–3. https://doi.org/10.1128/jb.182.23.6850-6853.2000.
34. Vergne I, Chua J, Lee HH, Lucas M, Belisle J, Deretic V. Mechanism of phagolysosome biogenesis block by viable mycobacterium tuberculosis. Proc Natl Acad Sci U S A. 2005;102:4033–8. https://doi.org/10.1073/pnas.0409716102.
35. Cowley S, Ko M, Pick N, Chow R, Downing KJ, Gordhan BG, Betts JC, Mizrahi V, Smith DA, Stokes RW, et al. The mycobacterium tuberculosis protein serine/threonine kinase PknG is linked to cellular glutamate/glutamine levels and is important for growth in vivo. Mol Microbiol. 2004;52:1691–702. https://doi.org/10.1111/j.1365-2958.2004.04085.x.
36. Quigley J, Hughitt VK, Velikovsky CA, Mariuzza RA, El-Sayed NM, Briken V. The Cell Wall lipid PDIM contributes to Phagosomal escape and host cell exit of mycobacterium tuberculosis. MBio. 2017;8 https://doi.org/10.1128/mBio.00148-17.
37. Schnettger L, Rodgers A, Repnik U, Lai RP, Pei G, Verdoes M, Wilkinson RJ, Young DB, Gutierrez MG. A Rab20-dependent membrane trafficking pathway controls M. tuberculosis replication by regulating phagosome spaciousness and integrity. Cell Host Microbe. 2017;21:619–628.e615. https://doi.org/10.1016/j.chom.2017.04.004.

38. de Jonge MI, Pehau-Arnaudet G, Fretz MM, Romain F, Bottai D, Brodin P, Honoré N, Marchal G, Jiskoot W, England P, et al. ESAT-6 from mycobacterium tuberculosis dissociates from its putative chaperone CFP-10 under acidic conditions and exhibits membrane-lysing activity. J Bacteriol. 2007;189:6028–34. https://doi.org/10.1128/jb.00469-07.
39. Houben D, Demangel C, van Ingen J, Perez J, Baldeón L, Abdallah AM, Caleechurn L, Bottai D, van Zon M, de Punder K, et al. ESX-1-mediated translocation to the cytosol controls virulence of mycobacteria. Cell Microbiol. 2012;14:1287–98. https://doi.org/10.1111/j.1462-5822.2012.01799.x.
40. De Leon J, Jiang G, Ma Y, Rubin E, Fortune S, Sun J. Mycobacterium tuberculosis ESAT-6 exhibits a unique membrane-interacting activity that is not found in its ortholog from non-pathogenic mycobacterium smegmatis. J Biol Chem. 2012;287:44184–91. https://doi.org/10.1074/jbc.M112.420869.
41. Conrad WH, Osman MM, Shanahan JK, Chu F, Takaki KK, Cameron J, Hopkinson-Woolley D, Brosch R, Ramakrishnan L. Mycobacterial ESX-1 secretion system mediates host cell lysis through bacterium contact-dependent gross membrane disruptions. Proc Natl Acad Sci U S A. 2017;114:1371–6. https://doi.org/10.1073/pnas.1620133114.
42. Gatfield J, Pieters J. Essential role for cholesterol in entry of mycobacteria into macrophages. Science. 2000;288:1647–50. https://doi.org/10.1126/science.288.5471.1647.
43. Jayachandran R, Sundaramurthy V, Combaluzier B, Mueller P, Korf H, Huygen K, Miyazaki T, Albrecht I, Massner J, Pieters J. Survival of mycobacteria in macrophages is mediated by coronin 1-dependent activation of calcineurin. Cell. 2007;130:37–50. https://doi.org/10.1016/j.cell.2007.04.043.
44. Brightbill HD, Libraty DH, Krutzik SR, Yang RB, Belisle JT, Bleharski JR, Maitland M, Norgard MV, Plevy SE, Smale ST, et al. Host defense mechanisms triggered by microbial lipoproteins through toll-like receptors. Science. 1999;285:732–6. https://doi.org/10.1126/science.285.5428.732.
45. Long R, Light B, Talbot JA. Mycobacteriocidal action of exogenous nitric oxide. Antimicrob Agents Chemother. 1999;43:403–5. https://doi.org/10.1128/aac.43.2.403.
46. Yu K, Mitchell C, Xing Y, Magliozzo RS, Bloom BR, Chan J. Toxicity of nitrogen oxides and related oxidants on mycobacteria: M. tuberculosis is resistant to peroxynitrite anion. Tuber Lung Dis. 1999;79:191–8. https://doi.org/10.1054/tuld.1998.0203.
47. O'Brien L, Carmichael J, Lowrie DB, Andrew PW. Strains of mycobacterium tuberculosis differ in susceptibility to reactive nitrogen intermediates in vitro. Infect Immun. 1994;62:5187–90. https://doi.org/10.1128/iai.62.11.5187-5190.1994.
48. Flesch IE, Kaufmann SH. Mechanisms involved in mycobacterial growth inhibition by gamma interferon-activated bone marrow macrophages: role of reactive nitrogen intermediates. Infect Immun. 1991;59:3213–8. https://doi.org/10.1128/iai.59.9.3213-3218.1991.
49. Chan J, Xing Y, Magliozzo RS, Bloom BR. Killing of virulent mycobacterium tuberculosis by reactive nitrogen intermediates produced by activated murine macrophages. J Exp Med. 1992;175:1111–22. https://doi.org/10.1084/jem.175.4.1111.
50. MacMicking JD, North RJ, LaCourse R, Mudgett JS, Shah SK, Nathan CF. Identification of nitric oxide synthase as a protective locus against tuberculosis. Proc Natl Acad Sci U S A. 1997;94:5243–8. https://doi.org/10.1073/pnas.94.10.5243.
51. Flynn JL, Scanga CA, Tanaka KE, Chan J. Effects of aminoguanidine on latent murine tuberculosis. J Immunol. 1998;160:1796–803.
52. Scanga CA, Mohan VP, Tanaka K, Alland D, Flynn JL, Chan J. The inducible nitric oxide synthase locus confers protection against aerogenic challenge of both clinical and laboratory strains of mycobacterium tuberculosis in mice. Infect Immun. 2001;69:7711–7. https://doi.org/10.1128/iai.69.12.7711-7717.2001.
53. Li Z, Kelley C, Collins F, Rouse D, Morris S. Expression of katG in mycobacterium tuberculosis is associated with its growth and persistence in mice and Guinea pigs. J Infect Dis. 1998;177:1030–5. https://doi.org/10.1086/515254.

54. Darwin KH, Ehrt S, Gutierrez-Ramos JC, Weich N, Nathan CF. The proteasome of mycobacterium tuberculosis is required for resistance to nitric oxide. Science. 2003;302:1963–6. https://doi.org/10.1126/science.1091176.
55. Underhill DM, Ozinsky A, Smith KD, Aderem A. Toll-like receptor-2 mediates mycobacteria-induced proinflammatory signaling in macrophages. Proc Natl Acad Sci U S A. 1999;96:14459–63. https://doi.org/10.1073/pnas.96.25.14459.
56. Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, Ochoa MT, Schauber J, Wu K, Meinken C, et al. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science. 2006;311:1770–3. https://doi.org/10.1126/science.1123933.
57. Liu PT, Stenger S, Tang DH, Modlin RL. Cutting edge: vitamin D-mediated human antimicrobial activity against mycobacterium tuberculosis is dependent on the induction of cathelicidin. J Immunol. 2007;179:2060–3. https://doi.org/10.4049/jimmunol.179.4.2060.
58. Yuk JM, Shin DM, Lee HM, Yang CS, Jin HS, Kim KK, Lee ZW, Lee SH, Kim JM, Jo EK. Vitamin D3 induces autophagy in human monocytes/macrophages via cathelicidin. Cell Host Microbe. 2009;6:231–43. https://doi.org/10.1016/j.chom.2009.08.004.
59. Fabri M, Stenger S, Shin DM, Yuk JM, Liu PT, Realegeno S, Lee HM, Krutzik SR, Schenk M, Sieling PA, et al. Vitamin D is required for IFN-gamma-mediated antimicrobial activity of human macrophages. Sci Transl Med. 2011;3:104ra102. https://doi.org/10.1126/scitranslmed.3003045.
60. Gutierrez MG, Master SS, Singh SB, Taylor GA, Colombo MI, Deretic V. Autophagy is a defense mechanism inhibiting BCG and mycobacterium tuberculosis survival in infected macrophages. Cell. 2004;119:753–66. https://doi.org/10.1016/j.cell.2004.11.038.
61. MacMicking JD, Taylor GA, McKinney JD. Immune control of tuberculosis by IFN-gamma-inducible LRG-47. Science. 2003;302:654–9. https://doi.org/10.1126/science.1088063.
62. Singh SB, Davis AS, Taylor GA, Deretic V. Human IRGM induces autophagy to eliminate intracellular mycobacteria. Science. 2006;313:1438–41. https://doi.org/10.1126/science.1129577.
63. Kumar D, Nath L, Kamal MA, Varshney A, Jain A, Singh S, Rao KV. Genome-wide analysis of the host intracellular network that regulates survival of mycobacterium tuberculosis. Cell. 2010;140:731–43. https://doi.org/10.1016/j.cell.2010.02.012.
64. Manzanillo PS, Ayres JS, Watson RO, Collins AC, Souza G, Rae CS, Schneider DS, Nakamura K, Shiloh MU, Cox JS. The ubiquitin ligase parkin mediates resistance to intracellular pathogens. Nature. 2013;501:512–6. https://doi.org/10.1038/nature12566.
65. Sakowski ET, Koster S, Portal Celhay C, Park HS, Shrestha E, Hetzenecker SE, Maurer K, Cadwell K, Philips JA. Ubiquilin 1 promotes IFN-γ-induced Xenophagy of mycobacterium tuberculosis. PLoS Pathog. 2015;11:e1005076. https://doi.org/10.1371/journal.ppat.1005076.
66. Ouimet M, Koster S, Sakowski E, Ramkhelawon B, van Solingen C, Oldebeken S, Karunakaran D, Portal-Celhay C, Sheedy FJ, Ray TD, et al. Mycobacterium tuberculosis induces the miR-33 locus to reprogram autophagy and host lipid metabolism. Nat Immunol. 2016;17:677–86. https://doi.org/10.1038/ni.3434.
67. Saini NK, Baena A, Ng TW, Venkataswamy MM, Kennedy SC, Kunnath-Velayudhan S, Carreño LJ, Xu J, Chan J, Larsen MH, et al. Suppression of autophagy and antigen presentation by mycobacterium tuberculosis PE_PGRS47. Nat Microbiol. 2016;1:16133. https://doi.org/10.1038/nmicrobiol.2016.133.
68. Tian T, Woodworth J, Sköld M, Behar SM. In vivo depletion of CD11c+ cells delays the CD4+ T cell response to mycobacterium tuberculosis and exacerbates the outcome of infection. J Immunol. 2005;175:3268–72. https://doi.org/10.4049/jimmunol.175.5.3268.
69. Wolf AJ, Desvignes L, Linas B, Banaiee N, Tamura T, Takatsu K, Ernst JD. Initiation of the adaptive immune response to mycobacterium tuberculosis depends on antigen production in the local lymph node, not the lungs. J Exp Med. 2008;205:105–15. https://doi.org/10.1084/jem.20071367.

70. Olmos S, Stukes S, Ernst JD. Ectopic activation of mycobacterium tuberculosis-specific CD4+ T cells in lungs of CCR7−/− mice. J Immunol. 2010;184:895–901. https://doi.org/10.4049/jimmunol.0901230.
71. Bhatt K, Hickman SP, Salgame P. Cutting edge: a new approach to modeling early lung immunity in murine tuberculosis. J Immunol. 2004;172:2748–51. https://doi.org/10.4049/jimmunol.172.5.2748.
72. Khader SA, Partida-Sanchez S, Bell G, Jelley-Gibbs DM, Swain S, Pearl JE, Ghilardi N, Desauvage FJ, Lund FE, Cooper AM. Interleukin 12p40 is required for dendritic cell migration and T cell priming after mycobacterium tuberculosis infection. J Exp Med. 2006;203:1805–15. https://doi.org/10.1084/jem.20052545.
73. Wolf AJ, Linas B, Trevejo-Nuñez GJ, Kincaid E, Tamura T, Takatsu K, Ernst JD. Mycobacterium tuberculosis infects dendritic cells with high frequency and impairs their function in vivo. J Immunol. 2007;179:2509–19. https://doi.org/10.4049/jimmunol.179.4.2509.
74. Ottenhoff TH, Kumararatne D, Casanova JL. Novel human immunodeficiencies reveal the essential role of type-I cytokines in immunity to intracellular bacteria. Immunol Today. 1998;19:491–4. https://doi.org/10.1016/s0167-5699(98)01321-8.
75. Jouanguy E, Altare F, Lamhamedi S, Revy P, Emile JF, Newport M, Levin M, Blanche S, Seboun E, Fischer A, et al. Interferon-gamma-receptor deficiency in an infant with fatal bacille Calmette-Guérin infection. N Engl J Med. 1996;335:1956–61. https://doi.org/10.1056/nejm199612263352604.
76. Newport MJ, Huxley CM, Huston S, Hawrylowicz CM, Oostra BA, Williamson R, Levin M. A mutation in the interferon-gamma-receptor gene and susceptibility to mycobacterial infection. N Engl J Med. 1996;335:1941–9. https://doi.org/10.1056/nejm199612263352602.
77. Dorman SE, Holland SM. Mutation in the signal-transducing chain of the interferon-gamma receptor and susceptibility to mycobacterial infection. J Clin Invest. 1998;101:2364–9. https://doi.org/10.1172/jci2901.
78. Jouanguy E, Lamhamedi-Cherradi S, Lammas D, Dorman SE, Fondanèche MC, Dupuis S, Döffinger R, Altare F, Girdlestone J, Emile JF, et al. A human IFNGR1 small deletion hotspot associated with dominant susceptibility to mycobacterial infection. Nat Genet. 1999;21:370–8. https://doi.org/10.1038/7701.
79. Dupuis S, Dargemont C, Fieschi C, Thomassin N, Rosenzweig S, Harris J, Holland SM, Schreiber RD, Casanova JL. Impairment of mycobacterial but not viral immunity by a germline human STAT1 mutation. Science. 2001;293:300–3. https://doi.org/10.1126/science.1061154.
80. Filipe-Santos O, Bustamante J, Haverkamp MH, Vinolo E, Ku CL, Puel A, Frucht DM, Christel K, von Bernuth H, Jouanguy E, et al. X-linked susceptibility to mycobacteria is caused by mutations in NEMO impairing CD40-dependent IL-12 production. J Exp Med. 2006;203:1745–59. https://doi.org/10.1084/jem.20060085.
81. Altare F, Durandy A, Lammas D, Emile JF, Lamhamedi S, Le Deist F, Drysdale P, Jouanguy E, Döffinger R, Bernaudin F, et al. Impairment of mycobacterial immunity in human interleukin-12 receptor deficiency. Science. 1998;280:1432–5. https://doi.org/10.1126/science.280.5368.1432.
82. Altare F, Lammas D, Revy P, Jouanguy E, Döffinger R, Lamhamedi S, Drysdale P, Scheel-Toellner D, Girdlestone J, Darbyshire P, et al. Inherited interleukin 12 deficiency in a child with bacille Calmette-Guérin and salmonella enteritidis disseminated infection. J Clin Invest. 1998;102:2035–40. https://doi.org/10.1172/jci4950.
83. Elloumi-Zghal H, Barbouche MR, Chemli J, Béjaoui M, Harbi A, Snoussi N, Abdelhak S, Dellagi K. Clinical and genetic heterogeneity of inherited autosomal recessive susceptibility to disseminated Mycobacterium bovis bacille calmette-guérin infection. J Infect Dis. 2002;185:1468–75. https://doi.org/10.1086/340510.
84. Picard C, Fieschi C, Altare F, Al-Jumaah S, Al-Hajjar S, Feinberg J, Dupuis S, Soudais C, Al-Mohsen IZ, Génin E, et al. Inherited interleukin-12 deficiency: IL12B genotype and clini-

cal phenotype of 13 patients from six kindreds. Am J Hum Genet. 2002;70:336–48. https://doi.org/10.1086/338625.
85. de Jong R, Altare F, Haagen IA, Elferink DG, Boer T, van Breda Vriesman PJ, Kabel PJ, Draaisma JM, van Dissel JT, Kroon FP, et al. Severe mycobacterial and salmonella infections in interleukin-12 receptor-deficient patients. Science. 1998;280:1435–8. https://doi.org/10.1126/science.280.5368.1435.
86. Altare F, Ensser A, Breiman A, Reichenbach J, Baghdadi JE, Fischer A, Emile JF, Gaillard JL, Meinl E, Casanova JL. Interleukin-12 receptor beta1 deficiency in a patient with abdominal tuberculosis. J Infect Dis. 2001;184:231–6. https://doi.org/10.1086/321999.
87. Caragol I, Raspall M, Fieschi C, Feinberg J, Larrosa MN, Hernández M, Figueras C, Bertrán JM, Casanova JL, Español T. Clinical tuberculosis in 2 of 3 siblings with interleukin-12 receptor beta1 deficiency. Clin Infect Dis. 2003;37:302–6. https://doi.org/10.1086/375587.
88. Fieschi C, Bosticardo M, de Beaucoudrey L, Boisson-Dupuis S, Feinberg J, Santos OF, Bustamante J, Levy J, Candotti F, Casanova JL. A novel form of complete IL-12/IL-23 receptor beta1 deficiency with cell surface-expressed nonfunctional receptors. Blood. 2004;104:2095–101. https://doi.org/10.1182/blood-2004-02-0584.
89. Flynn JL, Chan J, Triebold KJ, Dalton DK, Stewart TA, Bloom BR. An essential role for interferon gamma in resistance to mycobacterium tuberculosis infection. J Exp Med. 1993;178:2249–54. https://doi.org/10.1084/jem.178.6.2249.
90. Cooper AM, Dalton DK, Stewart TA, Griffin JP, Russell DG, Orme IM. Disseminated tuberculosis in interferon gamma gene-disrupted mice. J Exp Med. 1993;178:2243–7. https://doi.org/10.1084/jem.178.6.2243.
91. Cooper AM, Magram J, Ferrante J, Orme IM. Interleukin 12 (IL-12) is crucial to the development of protective immunity in mice intravenously infected with mycobacterium tuberculosis. J Exp Med. 1997;186:39–45. https://doi.org/10.1084/jem.186.1.39.
92. Feng CG, Jankovic D, Kullberg M, Cheever A, Scanga CA, Hieny S, Caspar P, Yap GS, Sher A. Maintenance of pulmonary Th1 effector function in chronic tuberculosis requires persistent IL-12 production. J Immunol. 2005;174:4185–92. https://doi.org/10.4049/jimmunol.174.7.4185.
93. Kak G, Raza M, Tiwari BK. Interferon-gamma (IFN-gamma): exploring its implications in infectious diseases. Biomol Concepts. 2018;9:64–79. https://doi.org/10.1515/bmc-2018-0007.
94. Szabo SJ, Kim ST, Costa GL, Zhang X, Fathman CG, Glimcher LH. A novel transcription factor, T-bet, directs Th1 lineage commitment. Cell. 2000;100:655–69. https://doi.org/10.1016/s0092-8674(00)80702-3.
95. Matsuyama M, Ishii Y, Yageta Y, Ohtsuka S, Ano S, Matsuno Y, Morishima Y, Yoh K, Takahashi S, Ogawa K, et al. Role of Th1/Th17 balance regulated by T-bet in a mouse model of Mycobacterium avium complex disease. J Immunol. 2014;192:1707–17. https://doi.org/10.4049/jimmunol.1302258.
96. Sullivan BM, Jobe O, Lazarevic V, Vasquez K, Bronson R, Glimcher LH, Kramnik I. Increased susceptibility of mice lacking T-bet to infection with mycobacterium tuberculosis correlates with increased IL-10 and decreased IFN-gamma production. J Immunol. 2005;175:4593–602. https://doi.org/10.4049/jimmunol.175.7.4593.
97. Redford PS, Boonstra A, Read S, Pitt J, Graham C, Stavropoulos E, Bancroft GJ, O'Garra A. Enhanced protection to mycobacterium tuberculosis infection in IL-10-deficient mice is accompanied by early and enhanced Th1 responses in the lung. Eur J Immunol. 2010;40:2200–10. https://doi.org/10.1002/eji.201040433.
98. Mogues T, Goodrich ME, Ryan L, LaCourse R, North RJ. The relative importance of T cell subsets in immunity and immunopathology of airborne mycobacterium tuberculosis infection in mice. J Exp Med. 2001;193:271–80. https://doi.org/10.1084/jem.193.3.271.
99. Behar SM, Dascher CC, Grusby MJ, Wang CR, Brenner MB. Susceptibility of mice deficient in CD1D or TAP1 to infection with mycobacterium tuberculosis. J Exp Med. 1999;189:1973–80. https://doi.org/10.1084/jem.189.12.1973.

100. Sousa AO, Mazzaccaro RJ, Russell RG, Lee FK, Turner OC, Hong S, Van Kaer L, Bloom BR. Relative contributions of distinct MHC class I-dependent cell populations in protection to tuberculosis infection in mice. Proc Natl Acad Sci U S A. 2000;97:4204–8. https://doi.org/10.1073/pnas.97.8.4204.
101. Chen CY, Huang D, Wang RC, Shen L, Zeng G, Yao S, Shen Y, Halliday L, Fortman J, McAllister M, et al. A critical role for CD8 T cells in a nonhuman primate model of tuberculosis. PLoS Pathog. 2009;5:e1000392. https://doi.org/10.1371/journal.ppat.1000392.
102. van Pinxteren LA, Cassidy JP, Smedegaard BH, Agger EM, Andersen P. Control of latent mycobacterium tuberculosis infection is dependent on CD8 T cells. Eur J Immunol. 2000;30:3689–98. https://doi.org/10.1002/1521-4141(200012)30:12<3689::Aid-immu3689>3.0.Co;2-4.
103. Stenger S, Mazzaccaro RJ, Uyemura K, Cho S, Barnes PF, Rosat JP, Sette A, Brenner MB, Porcelli SA, Bloom BR, et al. Differential effects of cytolytic T cell subsets on intracellular infection. Science. 1997;276:1684–7. https://doi.org/10.1126/science.276.5319.1684.
104. Stenger S, Hanson DA, Teitelbaum R, Dewan P, Niazi KR, Froelich CJ, Ganz T, Thoma-Uszynski S, Melián A, Bogdan C, et al. An antimicrobial activity of cytolytic T cells mediated by granulysin. Science. 1998;282:121–5. https://doi.org/10.1126/science.282.5386.121.
105. Ernst WA, Thoma-Uszynski S, Teitelbaum R, Ko C, Hanson DA, Clayberger C, Krensky AM, Leippe M, Bloom BR, Ganz T, et al. Granulysin, a T cell product, kills bacteria by altering membrane permeability. J Immunol. 2000;165:7102–8. https://doi.org/10.4049/jimmunol.165.12.7102.
106. Bruns H, Meinken C, Schauenberg P, Härter G, Kern P, Modlin RL, Antoni C, Stenger S. Anti-TNF immunotherapy reduces CD8+ T cell-mediated antimicrobial activity against mycobacterium tuberculosis in humans. J Clin Invest. 2009;119:1167–77. https://doi.org/10.1172/jci38482.
107. Shams H, Klucar P, Weis SE, Lalvani A, Moonan PK, Safi H, Wizel B, Ewer K, Nepom GT, Lewinsohn DM, et al. Characterization of a mycobacterium tuberculosis peptide that is recognized by human CD4+ and CD8+ T cells in the context of multiple HLA alleles. J Immunol. 2004;173:1966–77. https://doi.org/10.4049/jimmunol.173.3.1966.
108. Lalvani A, Brookes R, Wilkinson RJ, Malin AS, Pathan AA, Andersen P, Dockrell H, Pasvol G, Hill AV. Human cytolytic and interferon gamma-secreting CD8+ T lymphocytes specific for mycobacterium tuberculosis. Proc Natl Acad Sci U S A. 1998;95:270–5. https://doi.org/10.1073/pnas.95.1.270.
109. Pathan AA, Wilkinson KA, Wilkinson RJ, Latif M, McShane H, Pasvol G, Hill AV, Lalvani A. High frequencies of circulating IFN-gamma-secreting CD8 cytotoxic T cells specific for a novel MHC class I-restricted mycobacterium tuberculosis epitope in M. tuberculosis-infected subjects without disease. Eur J Immunol. 2000;30:2713–21. https://doi.org/10.1002/1521-4141(200009)30:9<2713::Aid-immu2713>3.0.Co;2-4.
110. Klein MR, Smith SM, Hammond AS, Ogg GS, King AS, Vekemans J, Jaye A, Lukey PT, McAdam KP. HLA-B*35-restricted CD8 T cell epitopes in the antigen 85 complex of mycobacterium tuberculosis. J Infect Dis. 2001;183:928–34. https://doi.org/10.1086/319267.
111. Caccamo N, Meraviglia S, La Mendola C, Guggino G, Dieli F, Salerno A. Phenotypical and functional analysis of memory and effector human CD8 T cells specific for mycobacterial antigens. J Immunol. 2006;177:1780–5. https://doi.org/10.4049/jimmunol.177.3.1780.
112. Lewinsohn DA, Winata E, Swarbrick GM, Tanner KE, Cook MS, Null MD, Cansler ME, Sette A, Sidney J, Lewinsohn DM. Immunodominant tuberculosis CD8 antigens preferentially restricted by HLA-B. PLoS Pathog. 2007;3:1240–9. https://doi.org/10.1371/journal.ppat.0030127.
113. Lewinsohn DM, Swarbrick GM, Cansler ME, Null MD, Rajaraman V, Frieder MM, Sherman DR, McWeeney S, Lewinsohn DA. Human mycobacterium tuberculosis CD8 T cell antigens/epitopes identified by a proteomic peptide library. PLoS One. 2013;8:e67016. https://doi.org/10.1371/journal.pone.0067016.

114. Carranza C, Pedraza-Sanchez S, de Oyarzabal-Mendez E, Torres M. Diagnosis for latent tuberculosis infection: new alternatives. Front Immunol. 2006;2020:11. https://doi.org/10.3389/fimmu.2020.02006.
115. Simmons JD, Stein CM, Seshadri C, Campo M, Alter G, Fortune S, Schurr E, Wallis RS, Churchyard G, Mayanja-Kizza H, et al. Immunological mechanisms of human resistance to persistent mycobacterium tuberculosis infection. Nat Rev Immunol. 2018;18:575–89. https://doi.org/10.1038/s41577-018-0025-3.
116. Behr MA, Edelstein PH, Ramakrishnan L. Revisiting the timetable of tuberculosis. BMJ. 2018;362:k2738. https://doi.org/10.1136/bmj.k2738.
117. Bloch H, Segal W. Biochemical differentiation of mycobacterium tuberculosis grown in vivo and in vitro. J Bacteriol. 1956;72:132–41. https://doi.org/10.1128/jb.72.2.132-141.1956.
118. Bishai W. Lipid lunch for persistent pathogen. Nature. 2000;406:683–5. https://doi.org/10.1038/35021159.
119. Boshoff HI, Myers TG, Copp BR, McNeil MR, Wilson MA, Barry CE 3rd. The transcriptional responses of mycobacterium tuberculosis to inhibitors of metabolism: novel insights into drug mechanisms of action. J Biol Chem. 2004;279:40174–84. https://doi.org/10.1074/jbc.M406796200.
120. American Thoracic Society/Centers for Disease Control and Prevention/Infectious Diseases Society of America: controlling tuberculosis in the United States. Am J Respir Crit Care Med. 2005;172:1169–227. https://doi.org/10.1164/rccm.2508001.
121. Herrera V, Perry S, Parsonnet J, Banaei N. Clinical application and limitations of interferon-gamma release assays for the diagnosis of latent tuberculosis infection. Clin Infect Dis. 2011;52:1031–7. https://doi.org/10.1093/cid/cir068.
122. Selwyn PA, Hartel D, Lewis VA, Schoenbaum EE, Vermund SH, Klein RS, Walker AT, Friedland GH. A prospective study of the risk of tuberculosis among intravenous drug users with human immunodeficiency virus infection. N Engl J Med. 1989;320:545–50. https://doi.org/10.1056/nejm198903023200901.
123. Selwyn PA, Sckell BM, Alcabes P, Friedland GH, Klein RS, Schoenbaum EE. High risk of active tuberculosis in HIV-infected drug users with cutaneous anergy. JAMA. 1992;268:504–9.
124. Markowitz N, Hansen NI, Hopewell PC, Glassroth J, Kvale PA, Mangura BT, Wilcosky TC, Wallace JM, Rosen MJ, Reichman LB. Incidence of tuberculosis in the United States among HIV-infected persons. The pulmonary complications of HIV infection study group. Ann Intern Med. 1997;126:123–32. https://doi.org/10.7326/0003-4819-126-2-199701150-00005.
125. Ehlers S. Role of tumour necrosis factor (TNF) in host defence against tuberculosis: implications for immunotherapies targeting TNF. Ann Rheum Dis. 2003;62 Suppl 2:ii37-42. https://doi.org/10.1136/ard.62.suppl_2.ii37.
126. Kalliolias GD, Ivashkiv LB. TNF biology, pathogenic mechanisms and emerging therapeutic strategies. Nat Rev Rheumatol. 2016;12:49–62. https://doi.org/10.1038/nrrheum.2015.169.
127. Lin PL, Plessner HL, Voitenok NN, Flynn JL. Tumor necrosis factor and tuberculosis. J Investig Dermatol Symp Proc. 2007;12:22–5. https://doi.org/10.1038/sj.jidsymp.5650027.
128. Johnson CM, Cooper AM, Frank AA, Orme IM. Adequate expression of protective immunity in the absence of granuloma formation in mycobacterium tuberculosis-infected mice with a disruption in the intracellular adhesion molecule 1 gene. Infect Immun. 1998;66:1666–70. https://doi.org/10.1128/iai.66.4.1666-1670.1998.
129. Schreiber T, Ehlers S, Aly S, Hölscher A, Hartmann S, Lipp M, Lowe JB, Hölscher C. Selectin ligand-independent priming and maintenance of T cell immunity during airborne tuberculosis. J Immunol. 2006;176:1131–40. https://doi.org/10.4049/jimmunol.176.2.1131.
130. Harris J, Keane J. How tumour necrosis factor blockers interfere with tuberculosis immunity. Clin Exp Immunol. 2010;161:1–9. https://doi.org/10.1111/j.1365-2249.2010.04146.x.
131. Harris J, Hope JC, Keane J. Tumor necrosis factor blockers influence macrophage responses to mycobacterium tuberculosis. J Infect Dis. 2008;198:1842–50. https://doi.org/10.1086/593174.

132. Harris J, Hope JC, Lavelle EC. Autophagy and the immune response to TB. Transbound Emerg Dis. 2009;56:248–54. https://doi.org/10.1111/j.1865-1682.2009.01069.x.
133. Di Sabatino A, Ciccocioppo R, Cinque B, Millimaggi D, Morera R, Ricevuti L, Cifone MG, Corazza GR. Defective mucosal T cell death is sustainably reverted by infliximab in a caspase dependent pathway in Crohn's disease. Gut. 2004;53:70–7. https://doi.org/10.1136/gut.53.1.70.
134. Mitoma H, Horiuchi T, Hatta N, Tsukamoto H, Harashima S, Kikuchi Y, Otsuka J, Okamura S, Fujita S, Harada M. Infliximab induces potent anti-inflammatory responses by outside-to-inside signals through transmembrane TNF-alpha. Gastroenterology. 2005;128:376–92. https://doi.org/10.1053/j.gastro.2004.11.060.
135. Shen C, Assche GV, Colpaert S, Maerten P, Geboes K, Rutgeerts P, Ceuppens JL. Adalimumab induces apoptosis of human monocytes: a comparative study with infliximab and etanercept. Aliment Pharmacol Ther. 2005;21:251–8. https://doi.org/10.1111/j.1365-2036.2005.02309.x.
136. Keane J, Gershon S, Wise RP, Mirabile-Levens E, Kasznica J, Schwieterman WD, Siegel JN, Braun MM. Tuberculosis associated with infliximab, a tumor necrosis factor alpha-neutralizing agent. N Engl J Med. 2001;345:1098–104. https://doi.org/10.1056/NEJMoa011110.
137. Gómez-Reino JJ, Carmona L, Valverde VR, Mola EM, Montero MD. Treatment of rheumatoid arthritis with tumor necrosis factor inhibitors may predispose to significant increase in tuberculosis risk: a multicenter active-surveillance report. Arthritis Rheum. 2003;48:2122–7. https://doi.org/10.1002/art.11137.
138. Brassard P, Kezouh A, Suissa S. Antirheumatic drugs and the risk of tuberculosis. Clin Infect Dis. 2006;43:717–22. https://doi.org/10.1086/506935.
139. Hsia EC, Schluger N, Cush JJ, Chaisson RE, Matteson EL, Xu S, Beutler A, Doyle MK, Hsu B, Rahman MU. Interferon-γ release assay versus tuberculin skin test prior to treatment with golimumab, a human anti-tumor necrosis factor antibody, in patients with rheumatoid arthritis, psoriatic arthritis, or ankylosing spondylitis. Arthritis Rheum. 2012;64:2068–77. https://doi.org/10.1002/art.34382.
140. Bykerk VP, Cush J, Winthrop K, Calabrese L, Lortholary O, de Longueville M, van Vollenhoven R, Mariette X. Update on the safety profile of certolizumab pegol in rheumatoid arthritis: an integrated analysis from clinical trials. Ann Rheum Dis. 2015;74:96–103. https://doi.org/10.1136/annrheumdis-2013-203660.
141. Ishida Y, Agata Y, Shibahara K, Honjo T. Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. EMBO J. 1992;11:3887–95.
142. Okazaki T, Honjo T. PD-1 and PD-1 ligands: from discovery to clinical application. Int Immunol. 2007;19:813–24. https://doi.org/10.1093/intimm/dxm057.
143. Shimatani K, Nakashima Y, Hattori M, Hamazaki Y, Minato N. PD-1+ memory phenotype CD4+ T cells expressing C/EBPalpha underlie T cell immunodepression in senescence and leukemia. Proc Natl Acad Sci U S A. 2009;106:15807–12. https://doi.org/10.1073/pnas.0908805106.
144. Keir ME, Butte MJ, Freeman GJ, Sharpe AH. PD-1 and its ligands in tolerance and immunity. Annu Rev Immunol. 2008;26:677–704. https://doi.org/10.1146/annurev.immunol.26.021607.090331.
145. Yamazaki T, Akiba H, Iwai H, Matsuda H, Aoki M, Tanno Y, Shin T, Tsuchiya H, Pardoll DM, Okumura K, et al. Expression of programmed death 1 ligands by murine T cells and APC. J Immunol. 2002;169:5538–45. https://doi.org/10.4049/jimmunol.169.10.5538.
146. Okazaki T, Honjo T. The PD-1-PD-L pathway in immunological tolerance. Trends Immunol. 2006;27:195–201. https://doi.org/10.1016/j.it.2006.02.001.
147. Barber DL, Wherry EJ, Masopust D, Zhu B, Allison JP, Sharpe AH, Freeman GJ, Ahmed R. Restoring function in exhausted CD8 T cells during chronic viral infection. Nature. 2006;439:682–7. https://doi.org/10.1038/nature04444.
148. Day CL, Kaufmann DE, Kiepiela P, Brown JA, Moodley ES, Reddy S, Mackey EW, Miller JD, Leslie AJ, DePierres C, et al. PD-1 expression on HIV-specific T cells is associated with

T-cell exhaustion and disease progression. Nature. 2006;443:350–4. https://doi.org/10.1038/nature05115.
149. Boni C, Fisicaro P, Valdatta C, Amadei B, Di Vincenzo P, Giuberti T, Laccabue D, Zerbini A, Cavalli A, Missale G, et al. Characterization of hepatitis B virus (HBV)-specific T-cell dysfunction in chronic HBV infection. J Virol. 2007;81:4215–25. https://doi.org/10.1128/jvi.02844-06.
150. Fujita K, Terashima T, Mio T. Anti-PD1 antibody treatment and the development of acute pulmonary tuberculosis. J Thorac Oncol. 2016;11:2238–40. https://doi.org/10.1016/j.jtho.2016.07.006.
151. Elkington PT, Bateman AC, Thomas GJ, Ottensmeier CH. Implications of tuberculosis reactivation after immune checkpoint inhibition. Am J Respir Crit Care Med. 2018;198:1451–3. https://doi.org/10.1164/rccm.201807-1250LE.
152. Barber DL, Sakai S, Kudchadkar RR, Fling SP, Day TA, Vergara JA, Ashkin D, Cheng JH, Lundgren LM, Raabe VN, et al. Tuberculosis following PD-1 blockade for cancer immunotherapy. Sci Transl Med. 2019;11:doi:10.1126/scitranslmed.aat2702.
153. Shi J, Li J, Wang Q, Cheng X, Du H, Han R, Li X, Zhao C, Gao G, He Y, et al. The safety and efficacy of immunotherapy with anti-programmed cell death 1 monoclonal antibody for lung cancer complicated with mycobacterium tuberculosis infection. Transl Lung Cancer Res. 2021;10:3929–42. https://doi.org/10.21037/tlcr-21-524.
154. Sakai S, Kauffman KD, Sallin MA, Sharpe AH, Young HA, Ganusov VV, Barber DL. CD4 T cell-derived IFN-γ plays a minimal role in control of pulmonary mycobacterium tuberculosis infection and must be actively repressed by PD-1 to prevent lethal disease. PLoS Pathog. 2016;12:e1005667. https://doi.org/10.1371/journal.ppat.1005667.
155. Lázár-Molnár E, Chen B, Sweeney KA, Wang EJ, Liu W, Lin J, Porcelli SA, Almo SC, Nathenson SG, Jacobs WR Jr. Programmed death-1 (PD-1)-deficient mice are extraordinarily sensitive to tuberculosis. Proc Natl Acad Sci U S A. 2010;107:13402–7. https://doi.org/10.1073/pnas.1007394107.
156. Lee J, Kornfeld H. Interferon-γ regulates the death of M. tuberculosis-infected macrophages. J Cell Death. 2010;3:1–11. https://doi.org/10.4137/jcd.s2822.
157. Tezera LB, Bielecka MK, Ogongo P, Walker NF, Ellis M, Garay-Baquero DJ, Thomas K, Reichmann MT, Johnston DA, Wilkinson KA, et al. Anti-PD-1 immunotherapy leads to tuberculosis reactivation via dysregulation of TNF-α. elife. 2020;9 https://doi.org/10.7554/eLife.52668.
158. Kauffman KD, Sakai S, Lora NE, Namasivayam S, Baker PJ, Kamenyeva O, Foreman TW, Nelson CE, Oliveira-de-Souza D, Vinhaes CL, et al. PD-1 blockade exacerbates mycobacterium tuberculosis infection in rhesus macaques. Sci Immunol. 2021;6 https://doi.org/10.1126/sciimmunol.abf3861.

Chapter 3
Impact of Biologic and JAK Inhibitor Therapies on TB: How Do Biologic Therapies Affect the Presentation and Treatment Course of Pulmonary TB?

Tomoshige Matsumoto

Abstract The biologics, such as Tumor Necrosis Factor (TNF) inhibitor, and Janus kinase (JAK) inhibitors have changed the treatment of autoimmune diseases, cancer, and various diseases. The JAK has two protein kinases, like Janus in Roman mythology. The biologics and JAK inhibitors have bright and somber sides. The somber is that it is prone to infectious diseases. The infection that develops depends on type of the drug. TB is more likely to occur with anti-TNF inhibitors, but less likely with JAK inhibitors. We report that endogenous JAK inhibitors such as Suppressor of Cytokine Signaling, SOCSs are present in our cells and that mice knocked out of SOCS cannot survive. JAK inhibitors provide brake assistance for endogenous JAK inhibitor such as SOCS. SOCS protein has been reported to suppress the development of TB. This is consistent with the fact that JAK inhibitors are less likely to develop TB than anti-TNF inhibitors. If TB develops with anti-TNF inhibitor, we showed that the anti-TNF inhibitor can be readministered after TB treatment. The pathophysiology of TB is due to an excessive immune response to *M. tuberculosis* cell components. Therefore, it is expected that the TB treatment period can be shortened by combining anti-TB treatment with a drug that lowers immunity. So, it is presumed that both anti-TNF inhibitor and JAK inhibitor can shorten the duration of TB treatment. This idea is consistent with the idea of using dexamethasone and JAK inhibitors such as baricitinib to treat COVID-19.

Keywords *M. tuberculosis* · Biologics · Anti-TNF inhibitor · JAK inhibitor · SOCS

T. Matsumoto (✉)
Osaka Fukujuji Hospital, Osaka Anti-Tuberculosis Association (Former: Osaka Hospital, Osaka Anti-Tuberculosis Association), Osaka, Japan
e-mail: tom_matsumoto@sutv.zaq.ne.jp

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1 Introduction

Recent developments in molecular biology have brought us a variety of new therapeutic methods. Molecular biology has revealed that various activities of life are carried out by cytokine signaling, and control of cytokine activity has led to the treatment of inflammatory diseases. Some of them include molecular-targeted therapies using biologics and JAK inhibitors. Molecular-targeted therapies have come to be used for various autoimmune inflammatory diseases. However, some problems have also been pointed out. For example, in 2001 Keane et al. announced that patients with rheumatoid arthritis who used the anti-TNF inhibitor infliximab had a higher incidence of disseminated TB than those who did not. It is also reported that disseminated TB was difficult to diagnose and there were more deaths than usual due to the delay in diagnosis. The package inserts state that administration of many biologics and JAK inhibitors to patients with active TB is contraindicated in Japan.

1.1 The Dawn of the Era of Biologics

In 2012, Humira ranked first in the world sales ranking, followed by Remicade in second place, Enbrel in fourth place, and Rituxan in fifth place (Note: not applicable in Japan) [1]. In this way, biologics used for rheumatoid arthritis are ranked high in the world sales ranking.

The frequency of TB development varies among biologics [2]. Biologics like anti-TNF inhibitors, anti-IL-6 inhibitors, and T cell function regulators are mainly used for rheumatoid arthritis. Anti-TNF inhibitors are used to treat Behcet's disease, and anti-TNF inhibitors, anti-IL17 inhibitors, and anti-IL23 inhibitors are used to treat psoriasis vulgaris and psoriatic arthritis. Anti-Blys antibodies are used in SLE, and it is said that these preparations other than T cell function regulators may exacerbate TB.

2 Pathophysiology of TB

TB is one of the three major infectious diseases in the world along with AIDS and malaria, and it is one of the infectious diseases for which future control issues still remain [3]. When described in this way, there is an image that TB is a highly virulent infectious disease. *Mycobacterium tuberculosis* (*M. tuberculosis*) is incapable of producing toxins. Proliferation in a host that is not immune to *M. tuberculosis* causes few symptoms. In fact, Yuichi Yamamura et al. have shown that injecting dead *M. tuberculosis* or the bacterial cell components of *M. tuberculosis* into the lungs of rabbits sensitized to *M. tuberculosis* causes cavity formation [4, 5]. In addition, they showed that cavities did not form even if the same thing was done for rabbits that were not sensitized to *M. tuberculosis*. From these results, they showed that the TB cavity is caused by an immune reaction against the cell component of

M. tuberculosis. In other words, TB is caused by the host's immune response to the TB cell component, and the pathophysiology of TB is determined by the amount of the cell component and the strength of the immune response to it [6], regardless of whether it is alive or dead.

3 TNF Inhibitors and TB

When *M. tuberculosis* invade the body, cells responsible for innate immunity such as macrophages surround the bacilli, engulf them and form granulation tissue [7]. TNF is involved in this granuloma formation. This condition is a latent TB infection [8].

3.1 Why Do TNF Inhibitors Make TB More Prone to Activation?

When an anti-TNF inhibitor is administered in the presence of granuloma surrounding *M. tuberculosis*, granulation cannot be maintained because there is no TNF. Moreover, membrane-type TNF is present on the cell surface of macrophage that phagocytoses or surrounds *M. tuberculosis* [7]. Since it is present, the anti-TNF antibody destroys macrophages and releases *M. tuberculosis* into the body [9, 10, 11, 12]. It is said that this causes TB reactivation.

3.2 Treatment of Latent TB Infections When RA Patients Are Administered with Biologics, Especially with Anti-TNF Inhibitors

Patients with latent TB infection should be treated for latent TB infection to administer Biologics, especially anti-TNF inhibitors. The following two ways are recommended (Fig. 3.1.): A method of performing anti-TNF inhibitor after conducting LTBI treatment with isoniazid for 6 months first [7]. And the LTBI treatment with isoniazid for 6 months is initiated 3 weeks before anti-TNF inhibitor administration [7].

The latter is more advantageous from the viewpoint of suppressing the reactivation of TB. This is because there are four types of tubercle bacilli in the body [13]: actively growing bacteria, semi-resting bacteria, resting bacteria, and dead bacteria. Isoniazid is only effective against actively growing bacteria. Therefore, even if LTBI treatment is performed first, semi-resting bacteria and resting bacteria may survive and be activated by anti-TNF inhibitors. There are several reports of TB after administration of anti-TNF inhibitors after LTBI treatment [14, 15]. When LTBI treatment is started 3 weeks before and then anti-TNF inhibitor administration

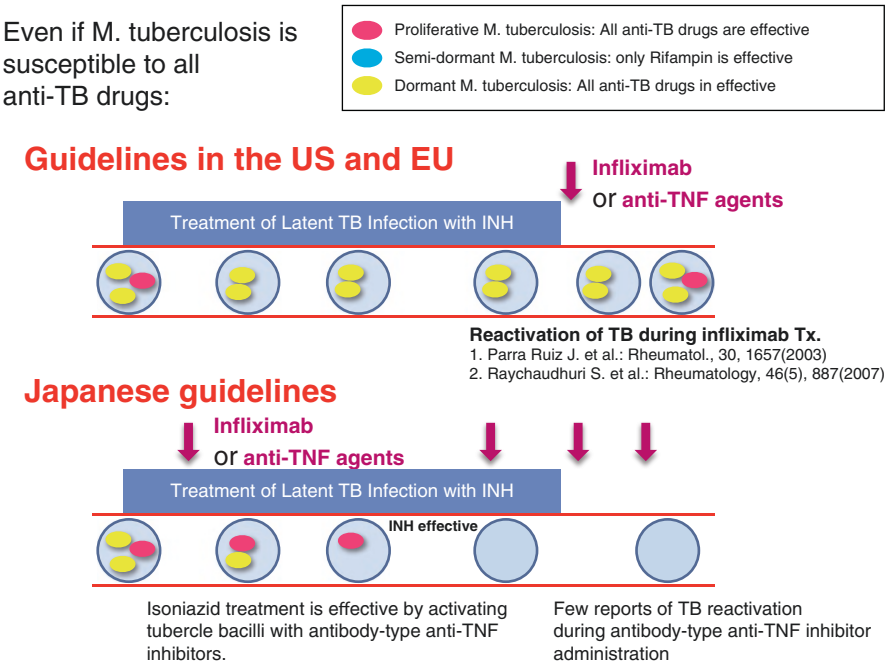

Fig. 3.1 Concept of LTBI treatment when anti-TNF inhibitor is administered

is started, the anti-TNF inhibitor activates semi-resting bacteria and resting bacteria. Therefore, LTBI treatment is more effective. In Japan, this latter method is used. However, this method does not mean that TB does not develop at all. When we examined all patients with rheumatoid arthritis who developed TB with the anti-TNF inhibitor adalimumab in Japan, some patients developed TB despite LTBI treatment [16]. These patients were more likely to develop the disease approximately 1 year after adalimumab administration [16]. The reason for the onset of TB is considered to be exotic reinfection, the blood concentration of INH did not rise and there was no effect even if it was taken [16], or it was not taken. Exotic reinfection of TB is seen in the high prevalence of TB, and the WHO LTBI guidelines for patients with HIV complicated TB in areas with high TB prevalence state that isoniazid should be taken for at least 36 months [17]. A Japanese statement was added to consider extending the treatment period for LTBI.

3.3 Differences in Prognosis Between Miliary TB and Pulmonary TB That Developed During Biologics Administration

We analyzed all Japanese patients with rheumatoid arthritis who developed TB after being treated with the anti-TNF inhibitor humira [16]. The prognosis was different between pulmonary TB and miliary TB, and there were no deaths or sequelae in

pulmonary TB, whereas deaths were observed in miliary TB [16] (Fig. 3.2). We compared the causes of rheumatoid arthritis patients who developed pulmonary TB and miliary TB by administration of humira, adalimumab, but there was no significant difference (Table 3.1). It should be noted that when comparing pulmonary TB and miliary TB, there was no difference in the period from the onset of symptoms to the start of treatment [16]. In other words, it was found that the delay in treatment did not make a difference in mortality.

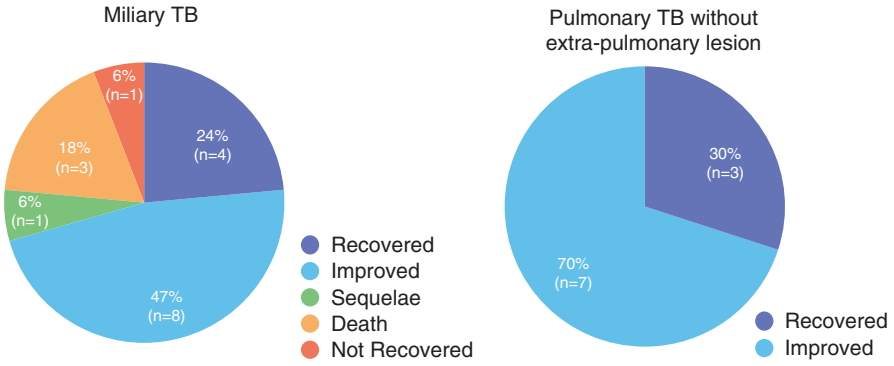

Fig. 3.2 The characteristics of TB caused by adalimumab are as follows: the first, 80% of number of TB was extrapulmonary TB. The second, there was no death of a person with pulmonary TB alone. The third, one-third of patients with extrapulmonary TB had severe adverse events, including death

Table 3.1 Factors predisposed to Miliary TB or Pulmonary TB in patients with rheumatoid arthritis who developed TB during administration of anti-TNF inhibitor, Adalimumab were compared and examined, but there was no significant difference

	Miliary TB (n=17)	Pulmonary TB (n=10)	p-value
Sex(male)	24.9%	70.0%	N.S.*2
Age	69.2 y.o.	63.6 y.o.	N.S.*2
Body weight(kg)	52.1kg	53.5kg	N.S.*1
With Complications	64.7%	50.0%	N.S.*2
No history of using Biologics	88.2%	60.0%	N.S.*2
Used in combination with MTX	70.6%	80.0%	N.S.*2
Used in combination with oral Corticosteroid	58.8%	60.0%	N.S.*2
Use with immunomodulators	5.9%	10.0%	N.S.*2
Period from the onset of illness to the start of medication(mean)	286.6 days	63.46 days	N.S.*1
The period from the initial symptoms to the start of treatment (mean)	36.9 days	30.4 days	N.S.*1

*1 Unpaired T-test *2 Fisher's exact test

3.4 *If Miliary TB Develops During Administration of a TNF Inhibitor, Discontinuation of the TNF Inhibitor Activates Immunity, Causes IRIS, and Worsens the Prognosis*

So what causes the death of a patient who develops TB during anti-TNF inhibitor administration?

Our analysis revealed the following: when TB develops in patients with rheumatoid arthritis who are receiving anti-TNF inhibitors, discontinuation of anti-TNF inhibitors enhances the immune response to *M. tuberculosis*. In the case of patients with pulmonary TB, even if the inflammation is enhanced, only the local response is enhanced, but in the case of miliary TB, the systemic inflammatory response is enhanced, that is, a so-called cytokine storm state (Fig. 3.3).

3.5 *Even if TB Develops during Administration of an Anti-TNF Inhibitor, It Is Possible to Readminister the Anti-TNF Inhibitor After TB Treatment*

What should we do for rheumatoid arthritis treatment after TB treatment in patients with rheumatoid arthritis who have developed TB with anti-TNF inhibitors?

We divided patients with rheumatoid arthritis with TB who received anti-TNF inhibitors into the following three groups and compared the sputum culture positive period (Fig. 3.4). Group A: The group that discontinued the anti-TNF inhibitor because the onset of TB was found, B Group: A group in which TB treatment was first performed and anti-TNF preparations were started in the middle, and Group C: A group in which administration of anti-TNF inhibitors was not discontinued even after the onset of TB. Group C had the shortest sputum culture positive period with a significant difference. And group A was the longest.

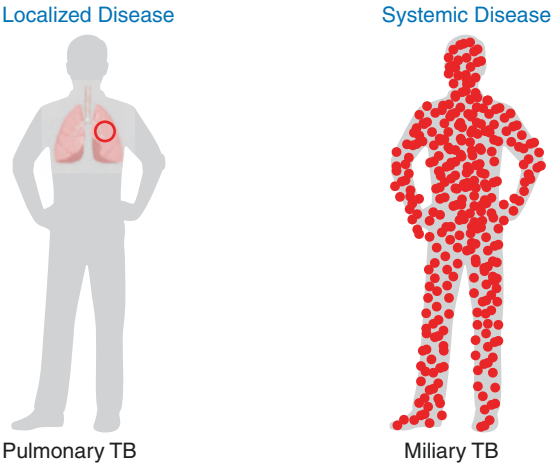

Fig. 3.3 Difference between miliary TB and pulmonary TB. Pulmonary TB is a localized disease, while miliary TB is a systemic disease

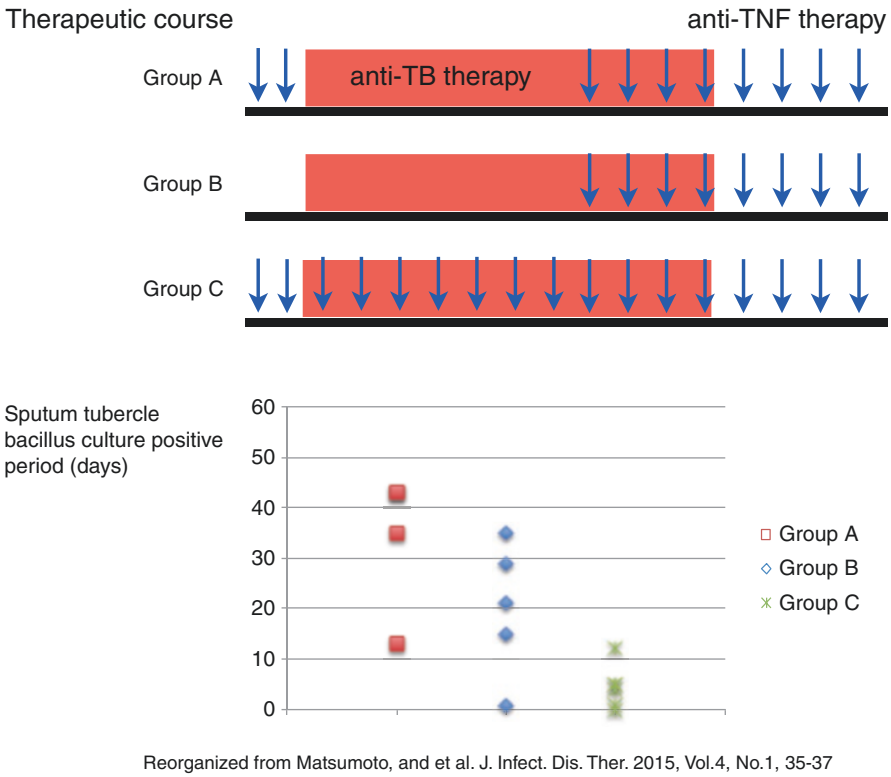

Fig. 3.4 Even if TB develops during the administration of an anti-TNF inhibitor, administration of the anti-TNF inhibitor can be continued if anti-TB drugs susceptible to *M. tuberculosis* are administered

Group C also had no IRIS during TB treatment and few subjective symptoms were reported.

If TB develops during administration of an anti-TNF inhibitor, there are two benefits to treating TB while continuing with the anti-TNF inhibitor: First, the anti-TNF inhibitor promotes the growth of *M. tuberculosis* and anti-TB drugs work more effectively. Second, inflammation is suppressed by anti-TNF inhibitors and paradoxical reaction does not occur [18].

3.6 Is It Possible to Readminister Anti-TNF Inhibitor After TB Treatment in Patients Who Develop Active TB during Anti-TNF Inhibitor Administration and Discontinue Anti-TNF Inhibitor?

When it was said that active TB was more likely to occur with anti-TNF inhibitor administration, it was believed that patients with rheumatism who developed TB with anti-TNF inhibitor could not be readministered with anti-TNF inhibitor even

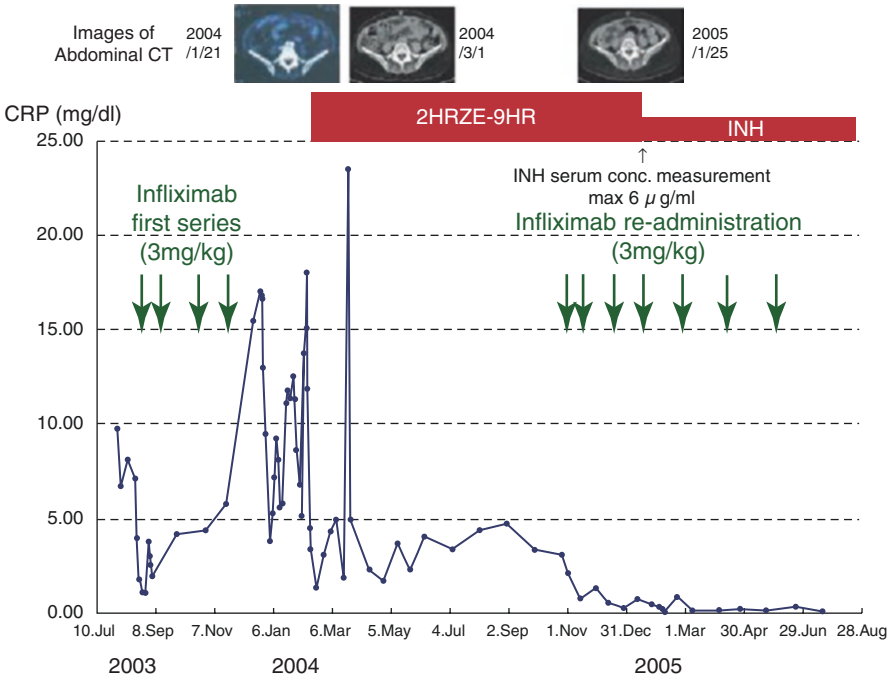

Fig. 3.5 A patient who developed peritoneal TB while receiving infliximab discontinued infliximab and started an anti-TB drug, but rheumatoid arthritis symptoms worsened and infliximab was readministered after the end of anti-TB treatment. Fifteen years after readministration of infliximab, no recurrence of TB has been observed

after TB treatment was completed. Such patients were not properly treated as their rheumatoid arthritis symptoms worsened. We have announced that patients with rheumatoid arthritis who develop active TB during anti-TNF inhibitor administration and who have completed anti-TB treatment can be readministered with anti-TNF inhibitor and can be treated with anti-TNF inhibitor without recurrence of TB [19] (Fig. 3.5). Currently, various guidelines state that even if active TB develops during administration of anti-TNF inhibitor, readministration of anti-TNF inhibitor is possible during or after TB treatment [20, 21].

4 JAK Inhibitor

Homeostasis in the body is maintained by various hormones, cytokines, and other substances. The production of these substances is precisely controlled under normal conditions. The amount of hormone produced is maintained by a feedback mechanism. The signal transduction system also has a negative feedback mechanism in

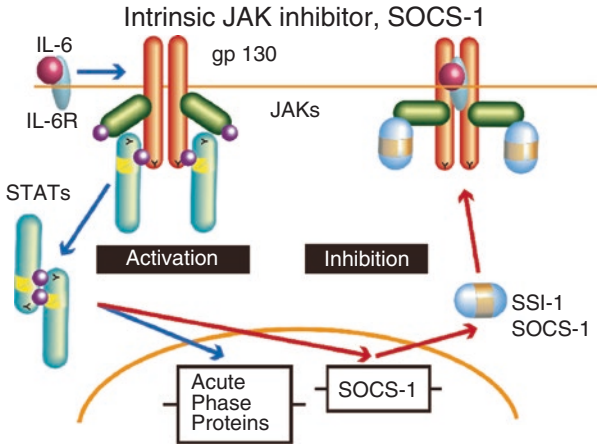

Fig. 3.6 There is a negative feedback mechanism in the cytokine intracellular signal transduction system. We are trying to isolate a family protein of STAT that plays a major role in the JAK-STAT system, which is an IL-6 intracellular signal transduction system, and we try to isolate a protein whose amino acid sequence is similar to STAT but does not signal. This protein is later called SOCS-1, and is a negative feedback factor of the JAK-STAT system that is induced via the JAK-STAT system by the IL-6 signal and suppresses the JAK-STAT system by binding to JAK. JAK inhibitors used in inflammatory diseases such as rheumatoid arthritis and COVID-19 have been reported to support the function of the endogenous JAK inhibitor SOCS

cells, and the JAK-STAT-SOCS system announced by us is a typical system [22]. The name of JAK comes from the god Janus. In an intracellular signal transduction system that utilizes the JAK-STAT system, when cytokines bind to receptors on the cell surface, JAK is activated and phosphorylates STAT. Activated STAT translocates from the cytoplasm into the nucleus, binds to DNA, and participates in messenger RNA (mRNA) synthesis as a transcription factor. We have announced that there is a Negative feedback mechanism in which SOCS mRNA is included in the transcribed mRNA and finally the synthesized SOCS protein binds to JAK and suppresses JAK activation [22, 23] (Fig. 3.6).

SOCS has seven family proteins [24]. Each SOCS has a unique ability to bind to the JAK protein at physiological concentrations. However, in the forced expression system, it binds to all JAK proteins and completely suppresses JAK function.

We generated SOCS-1-deficient mice. SOCS-1-deficient mice are born normally but develop failure to thrive and all individuals die within 1 month [25]. It has been reported that SOCS-3-deficient mice die in utero and are not born [26, 27] (Fig. 3.7). Therefore, SOCS, which suppresses JAK-STAT signaling, is an essential protein for vital activity, and it is presumed that death due to SOCS-1 deficiency is due to overstimulation of cytokine signals. In addition to the SOCS-1 protein, there are phosphotyrosine-phosphatase and Protein Inhibitor of Activated STAT (PIAS) proteins that suppress the function of JAK [28]. It has three braking mechanisms for the JAK-STAT system, which is the accelerator of the intracellular signal transduction system.

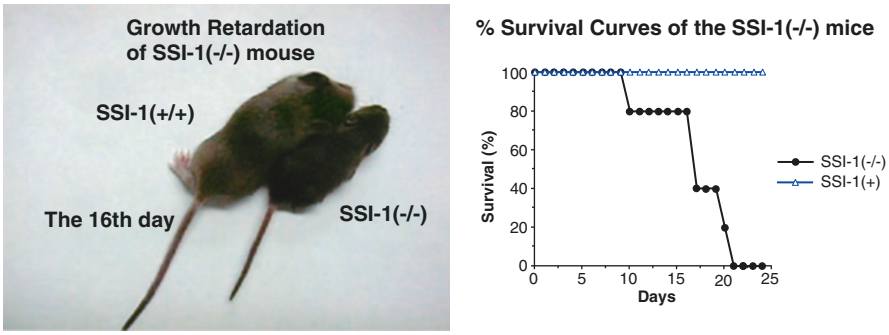

Fig. 3.7 SSI-1 deficient mice lacking SOCS-1 function are born normally, but develop failure to thrive and all individuals die within 1 month. This indicates that endogenous JAK inhibitors are essential substances for survival

JAK inhibitors have been used for rheumatoid arthritis, ulcerative colitis, and atopic dermatitis [29]. Administration of ts-DMARD tofacitinib suppresses phosphorylation of STAT and suppresses expression of SOCS. Tofacitinib suppresses cytokine intracellular signaling mechanisms with the same mechanism as SOCS, that is, Jak Inhibitor, such as Tofacitinib, has compensates for the action of endogenous Jak Inhibitor. Morelli etc. describe that the function of TOFACITINIB mimics the functions of SOCS-1 and SOCS-3 [30].

From a series of studies on the SOCS protein, when excessive inflammation occurs, the endogenous JAK inhibitor, SOCS is induced to try to suppress inflammation. However, when the SOCS protein cannot suppress excessive inflammation, oral JAK inhibitors are used to suppress persistent inflammation that SOCS cannot suppress. Oral JAK inhibitors are used as brake assists for SOCS proteins.

4.1 Endogenenous JAK inhibitors, SOCSs, and TB

Overproduction of the SOCS protein has been reported to reduce resistance to herpesviruses [31]. On the other hand, it is said that the production of SOCS protein increases resistance to tubercle bacilli [31, 32].

4.2 JAK Inhibitors and TB

Initially, it was reported that JAK inhibitors, like anti-TNF inhibitors, have reduced resistance to *M. tuberculosis* and are more likely to develop TB [33]. Administration to TB is contraindicated in the package insert of JAK inhibitors. However, there is no report that the incidence of TB increased due to the administration of JAK inhibitors in the clinical trials [34] and in the post-marketing surveillance in Japan [35]. Now, there is a widespread consensus that administration of JAK inhibitors does not increase the incidence of TB.

4.3 JAK Inhibitors and TB Therapy

It has been reported that the use of JAK inhibitors during the treatment of active TB makes it easier to kill tubercle bacilli in the body [36]. If TB develops during administration of a JAK inhibitor, prompt introduction of anti-TB drugs can lead to successful anti-TB treatment without discontinuing the JAK inhibitor.

5 Host-Directed TB Therapy

Immunosuppressive drug administration was often contraindicated in the treatment of infectious diseases.

However, with the use of dexamethasone and the JAK inhibitor, baricitinib, in the treatment of COVID-19, the way of thinking about infectious disease treatment is changing.

The same concept as COVID-19 treatment applies to TB treatment. Pathogenicity of TB is not toxin production but an excessive immune response to the cell component of TB but also plays a major role. The agents that are easy to develop TB cause TB proliferation by reducing TB immunity. Current anti-TB treatment is efficient in the proliferative TB bacteria, even under circumstances where the immunity to TB is reduced [18]. As the immunity activates and the growth of TB is reduced, the effect of the anti-TB drug is lowered [13].

Host-Directed Therapy is a method of enabling anti-TB treatment by adjusting host immunity [37, 38]. Many Host-Directed Therapy attempts are methods of reducing host immunity and shortening the treatment period of TB. We showed that using anti-TNF inhibitors for TB treatment and showed that the sputum culture positive period can be shortened [18], but Wallis, etc. propose the following drug as a drug used for Host-Directed Therapy other than anti-TNF inhibitors Corticosteroids, statin, thalidomide analogues, Phosphodiesterase, Cyclooxygenase, and Leukotrienes inhibitors [38]. In addition to the above agents, it was reported that if a high dose of tofacitinib is used in combination with anti-TB drugs, the TB treatment period may be shortened.

6 Conclusion

The pathogenicity of *M. tuberculosis* is not the production of toxins, but the excessive immune response to *M. tuberculosis* components plays a major role. Drugs that are prone to develop TB leads to the growth of *M. tuberculosis* by lowering TB immunity. Current anti-TB standard chemotherapy works efficiently against *M. tuberculosis* in the proliferative phase even if the immunity against *M. tuberculosis* is weakened, but when the immunity is activated and the growth of *M.*

tuberculosis is reduced, the anti-TB drug The bactericidal action is reduced. Therefore, under effective anti-TB drugs, drugs that reduce immunity and promote the growth of *M. tuberculosis* may be useful as an adjunct to anti-TB drugs.

References

1. Ben H. Abbott drug tops sales as Lipitor, Plavix era ends. reuters. 2012;
2. Landry J, Menzies D. State of the art state of the art series tuberculosis. In: Rusen ID, editor. The series preventive chemotherapy. Where has it got us? Where to go next? vol. 12. 2nd ed; 2008. http://www.meakins.mcgill.ca/meakins/NEW.
3. Global Tuberculosis Programme WHO. Latent tuberculosis infection updated and consolidated guidelines for programmatic management; 2018.
4. Yamamura Y. The pathogenesis of tuberculous cavities. Advn Tuberc Res. 1958;9:13–37.
5. Yamamura Y, Yasaka S, Nakamura S, et al. Experimental studies on the tuberculous allergy. III. Experimental formation of the tuberculous cavity in the rabbit's lung by killed tubercle bacillus. Kekkaku. 1954;29(9):361–7. English abstract, 376-377
6. Casadevall A, Pirofski LA. The damage-response framework of microbial pathogenesis. Nat Rev Microbiol. 2003;1(1):17–24. https://doi.org/10.1038/nrmicro732.
7. Suda T, Kameda H, Tasaka T, et al. Consensus statements for medical practice: biological agents for inflammatory diseases and lung disease (The Japanese Respiratory Society). 2020.
8. Cooper A. Cell-mediated immune responses in tuberculosis. Annu Rev Immunol. 2009;27(1):393–422.
9. Ehler S. Tumor necrosis factor and its blockade in granulomatous infections: differential modes of action of infliximab and etanercept? Clin Infect Dis. 2005;1(41 Supple 3):199–203.
10. Gardam M, Keystone E, Menzies R, et al. Anti-tumour necrosis factor agents and tuberculosis risk: mechanisms of action and clinical management. Lancet Infect Dis. 2003;3(3):148–55.
11. Ehlers S. Role of tumour necrosis factor (TNF) in host defence against tuberculosis: implications for immunotherapies targeting TNF. Ann Rheum Dis. 2003;62(Suppl 2):ii37–42.
12. Jacobs M, Samarina A, Grivennikov S, et al. Reactivation of tuberculosis by tumor necrosis factor neutralization. Eur Cytokine Netw. 2007;18(1):5–13. Epub 2007 Mar 30
13. Mitchison DA. Basic mechanisms of chemotherapy. Chest. 1979;76(6 Suppl):771–81. https://doi.org/10.1378/chest.76.6_supplement.771.
14. Parra Ruiz J, Centeno NO, Raya AE. Prophylactic Therapy with Isoniazid. Development of Tuberculosis in a Patient Treated with Infliximab Who Had Received, vol. 30; 2003. http://www.jrheum.org/content/30/7/1657.citation; http://www.jrheum.org/cgi/alerts/etoc; http://jrheum.com/subscribe.html; http://jrheum.com/reprints.html; www.jrheum.org; www.jrheum.org.downloadedfrom
15. Raychaudhuri S, Shmerling R, Ermann J, Helfgott S. Development of active tuberculosis following initiation of infliximab despite appropriate prophylaxis [3]. Rheumatology. 2007;46(5):887–8. https://doi.org/10.1093/rheumatology/kel447.
16. Igari H, Sakatani M, Matsumoto T, Watanabe A. Humira safety information post-marketing evaluation of tuberculosis cases. 2015.
17. WHO. Recommendation on 36 months isoniazid preventive therapy to adults and adolescents living with HIV in resource-constrained and high TB- and HIV-prevalence settings 2015 update. Geneva; 2015.
18. Matsumoto T, Tanaka T. Continuation of anti-TNF therapy for rheumatoid arthritis in patients with active tuberculosis reactivated during anti-TNF medication is more beneficial than its cessation. J Infect Dis Ther. 2015;3(1):35–7. https://doi.org/10.14205/2310-9386.2015.03.01.6.
19. Matsumoto T, Tanaka T, Kawase I. Infliximab for rheumatoid arthritis in a patient with tuberculosis. N Engl J Med. 2006;355(7):740–1. https://doi.org/10.1056/NEJMc053468.

20. Singh J, Furst D, Bharat A, et al. 2012 update of the 2008 American College of Rheumatology recommendations for the use of disease- modifying antirheumatic drugs and biologic agents in the treatment of rheumatoid arthritis. Arthritis Care Res. 2012;64(5):625–39.
21. Ding T, Ledingham J, Luqmani R, et al. BSR and BHPR rheumatoid arthritis guidelines on safety of anti-TNF therapies. Rheumatology. 2010;49(11):2217–9.
22. Naka T, Narazaki M, Hirata M, et al. Structure and function of a new STAT-induced STAT inhibitor. Nature. 1997;387(6636):924–9. https://doi.org/10.1038/43219.
23. Narazaki M, Fujimoto M, Matsumoto T, et al. Three distinct domains of SSI-1/SOCS-1/JAB protein are required for its suppression of interleukin 6 signaling. Proc Natl Acad Sci U S A. 1998;95(22):13130–4. https://doi.org/10.1073/pnas.95.22.13130.
24. Minamoto S, Ikegame K, Ueno K, et al. Cloning and functional analysis of new members of STAT induced STAT inhibitor (SSI) family: SSI-2 and SSI-3. Biochem Biophys Res Commun. 1997;237(1):79–83. https://doi.org/10.1006/bbrc.1997.7080.
25. Naka T, Matsumoto T, Narazaki M, et al. Accelerated apoptosis of lymphocytes by augmented induction of Bax in SSI-1 (STAT-induced STAT inhibitor-1) deficient mice. Proc Natl Acad Sci U S A. 1998;95(26):15577–82. https://doi.org/10.1073/pnas.95.26.15577.
26. Roberts AW, Robb L, Rakar S, et al. Placental defects and embryonic lethality in mice lacking suppressor of cytokine signaling. Proc Natl Acad Sci U S A. 98(16):9324–9. https://doi.org/10.1073/pnas.161271798.
27. Marine JC, McKay C, Wang D, et al. SOCS3 is essential in the regulation of fetal liver erythropoiesis. Cell. 1999;98(5):617–27. https://doi.org/10.1016/s0092-8674(00)80049-5.
28. Niu GJ, Xu JD, Yuan WJ, et al. Protein inhibitor of activated STAT (PIAS) negatively regulates the JAK/STAT pathway by inhibiting STAT phosphorylation and translocation. Front Immunol. 2018;9(OCT):2392. https://doi.org/10.3389/fimmu.2018.02392.
29. Fragoulis GE, Mcinnes IB, Siebert S, JAK-inhibitors. New players in the field of immune-mediated diseases, beyond rheumatoid arthritis. Rheumatol (UK). 2019;58:i43–54. https://doi.org/10.1093/rheumatology/key276.
30. Malemud CJ. Negative regulators of JAK/STAT signaling in rheumatoid arthritis and osteoarthritis. Int J Mol Sci. 2017;18(3):484. https://doi.org/10.3390/ijms18030484.
31. Carow B, Rottenberg ME. SOCS3, a major regulator of infection and inflammation. Front Immunol. 2014;5:58. https://doi.org/10.3389/fimmu.2014.00058.
32. Carow B, Reuschl A-K, Gavier-Widen D, et al. Critical and independent role for SOCS3 in either myeloid or T cells in resistance to mycobacterium tuberculosis. PLoS Pathog. 2013;9(7):e1003442. https://doi.org/10.1371/journal.ppat.1003442.
33. Winthrop KL, Park SH, Gul A, et al. Tuberculosis and other opportunistic infections in tofacitinib-treated patients with rheumatoid arthritis. Ann Rheum Dis. 2016;75(6):1133–8. https://doi.org/10.1136/annrheumdis-2015-207319.
34. Harigai M, Takeuchi T, Smolen JS, et al. Safety profile of baricitinib in Japanese patients with active rheumatoid arthritis with over 1.6 years median time in treatment: an integrated analysis of phases 2 and 3 trials. Mod Rheumatol. 2020;30(1):36–43. https://doi.org/10.1080/14397595.2019.1583711.
35. Harigai M. Growing evidence of the safety of JAK inhibitors in patients with rheumatoid arthritis. Rheumatol (UK). 2019;58:i34–42. https://doi.org/10.1093/rheumatology/key287.
36. Maiga M, Ahidjo BA, Maiga MC, et al. Efficacy of adjunctive Tofacitinib therapy in mouse models of tuberculosis. EBioMedicine. 2015;2(8):868–73. https://doi.org/10.1016/j.ebiom.2015.07.014.
37. Kaufmann SHE, Lange C, Rao M, et al. Progress in tuberculosis vaccine development and host-directed therapies–a state of the art review. Lancet Respir Med. 2014;2(4):301–20. https://doi.org/10.1016/S2213-2600(14)70033-5.
38. Wallis RS, Hafner R. Advancing host-directed therapy for tuberculosis. Nat Rev Immunol. 2015;15(4):255–63. https://doi.org/10.1038/nri3813.

Chapter 4
Advances in Mycobacterial Laboratories: What Is the Latest Laboratory Approach to Diagnose and Manage Pulmonary TB?

Satoshi Mitarai

Abstract Laboratory examination is one of the key factors in the management and diagnosis of TB as well as for, follow-up and decision to cure. So far, we have been using conventional smear microscopy and culture techniques; however, the situation has been changing in the last decade. As expected, conventional technologies are shifting to molecular-based technologies. Given the rapid nature of DNA-based molecular technologies, the turn-around time of reporting results, especially in *Mycobacterium tuberculosis* detection/identification and drug susceptibility testing, has reduced. Other technologies, such as, antigen detection using specimens other than sputum and the use of biomarkers (lipoarabinomannan, ESAT-6/CFP-10, and MPT64), have also become available in clinical practices. The new laboratory examinations have many advantages, although new problems have also emerged. In-depth knowledge of the merits and demerits of the new laboratory examinations is warranted, which will provide useful information if we can realize the details of each technology.

Keywords Concentration · Nucleic acid amplification · Antigen detection Whole-genome sequence

1 Introduction

Identification of *Mycobacterium tuberculosis* (Mtb) is an essential requirement for a definite diagnosis of TB. The presence of Mtb is evidenced by the detection of whole bacteria, specific nucleic acid sequences, and specific antigens of Mtb. Several techniques have been developed in the past century for the detection of Mtb

S. Mitarai (✉)
Department of Mycobacterium Reference and Research, The Research Institute of Tuberculosis, Japan Anti-Tuberculosis Association, Tokyo, Japan
e-mail: mitarai@jata.or.jp

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and/or its specific components. The most primitive method is smear microscopy with appropriate staining methods; however, acid-fast bacilli detected under a microscope require species identification because there are 193 officially registered mycobacterium species as of May 2021, and non-TB mycobacteriosis [1], a disease caused by non-tuberculosis mycobacterium (NTM), is generally common in many industrialized countries [2]. Thus, specific detection of Mtb is important for the accurate diagnosis of active TB. Mtb-specific laboratory examination methods have been developed over the last three decades. One objective of this chapter is to introduce and explain these methods.

One major problem for sensitive Mtb detection in clinical practice is the quality of the specimens for laboratory tests. In this case, quality refers to the concentration of Mtb measured in a certain unit, such as per mL. The complete dependence of detection sensitivity on the concentration of Mtb, not the absolute number, has not been well described. For example, a proportion of Mtb bacilli of 10,000 CFU/mL can be sufficient for detection even using smear microscopy. However, if they are dispersed in 100 mL, 100 CFU/mL concentration is critical, even when using the nucleic acid amplification (NAA) method. When one NAA utilizes 200 μL of the sample, it results in 20 Mtb bacilli in the starting volume. If only 25% of the sample is finally used for DNA amplification, only five target Mtb bacilli are present in the reaction tube. This is sometimes considered the limit of detection (LoD) for NAA. Therefore, it is important to obtain high-quality clinical specimens and develop efficient concentration methods. Methods for obtaining a good quality specimen are key to sensitive Mtb detection and diagnosis [3].

2 Clinical Specimen Collection

Sputum remains a major clinical specimen for the diagnosis of TB because approximately 80% of TB forms pulmonary disease. Many previous reports showed that high-quality sputum specimens yield highly positive results [4–6]. The World Health Organization (WHO) generally recommends the instructions for sputum expectoration for presumed patients with TB [7]. This procedure works in many cases; however, some patients, including young children, elderly people, and those with minimal lung lesions, have difficulty expectorating sufficient and good quality sputum [8]. Efficient technologies for collecting useful clinical specimens, mainly sputum, are important.

Lung Flute® is a US-FDA-registered musical instrument that helps the expectoration of sputum with acoustic resonance. Sakashita et al. reported the performance of Lung Flute® by head-to-head comparison of Lung Flute® and the hypertonic saline inhalation method. They clarified that there was no significant difference in the conversion rates between the two methods [9]. In many resource-limited settings, wherein no stable electricity is expected, Lung Flute® can be useful for collecting high-quality sputum specimens.

Face mask sampling is a new method for collecting Mtb bacilli from exhaled breath. The exhalation contains bioaerosolized Mtb, which may be coated with biomaterials such as sugars, lipids, and an alveolar surfactant. The Mtb bioaerosol is disseminated through coughing, talking, or singing. Thus, face masks are an effective way to collect Mtb bacilli. Williams et al. reported face mask sampling for Mtb detection. They tested the FFP30 mask modified to contain a gelatin filter for 1 h and detected 65% (13/20) of patients with active TB using the Xpert MTB/RIF [10]. This could be utilized to collect clinical specimens for the diagnosis of TB, especially in resource-limited settings. The following relatively large-scale study showed 86% (166/192) of Mtb detection using the face mask method and a polyvinyl alcohol sampling matrix as well as Xpert MTB/RIF Ultra [11].

Another method for the collection of bioaerosols from exhalation was developed by Tohoku University, although it is still at the press-release level. Akaike et al. reported that the aerosol collection system cooled down the exhalation and obtained cohesion from natural breath. The basic idea (cold cohesion) is not new, but the following omics analyses could provide various information, including the composition and viability of microorganisms in the specimen. Regarding TB, it could be a possible solution for efficient Mtb collection.

The WHO recently published a consolidated guideline on TB, module 3: diagnosis, and has recommended the following: "in children with signs and symptoms of pulmonary TB, Xpert MTB/RIF should be used as an initial diagnostic test for TB and rifampicin resistance detection in sputum, gastric aspirate, nasopharyngeal aspirate and stool rather than smear microscopy/culture and phenotypic DST. (Strong recommendation, moderate certainty for accuracy in sputum; low certainty of evidence for test accuracy in gastric aspirate, nasopharyngeal aspirate, and stool)" [12].

Gastric aspirate specimens showed sensitivity (67.5–91.4%) and specificity (89.6–99.3%) in several studies for the diagnosis of pediatric TB using the Xpert MTB/RIF or Ultra [13–15]. The diagnosis of pediatric TB using gastric aspirate is reliable, with relatively high specificity. However, the gastric aspirate collection process is invasive and somewhat dangerous and can injure the esophageal, gastric, or respiratory systems.

The nasopharyngeal aspirate is also a potential material for the diagnosis of TB. Owens et al. reported a diagnostic sensitivity of 62% and specificity of 98% among 438 patients using RealArt *M. tuberculosis* RT-PCR kit (Artus, Hamburg, Germany) [16]. Zar et al. reported the results of nasopharyngeal isolates from 535 patients and showed 65% sensitivity (including that in HIV-infected patients) and 98.2% specificity using Xpert MTB/RIF [17]. The performance of nasopharyngeal aspirates in their study showed relatively lower sensitivity than that of induced sputum specimens but almost the same specificity, which indicates the potential capacity of nasopharyngeal aspirate specimens in diagnosing pediatric TB.

Feces is a potentially important specimen for the diagnosis of respiratory TB because the spontaneously swollen sputum contains Mtb paths through the intestinal system and is discharged in it. Because Mtb are relatively resistant to acid and other stresses in the intestinal system, they survive and persist in the feces. However, feces contain many other bacteria; thus, the conventional culture technique with

ordinary pretreatment method is not efficient enough to recover Mtb with high efficiency. Oramasionwu et al. reported the performance of culture examination (liquid or solid) of feces for the diagnosis of TB in Cambodia and reported 44% recovery of Mtb (101/228) with 14.1% contamination results among TB patients living with HIV [18]. Walters E et al. reported a similar study with 4% culture positives and 41% of contaminations [19]. The NAA method can be useful for detecting the presence of Mtb DNA in a specimen, especially in the case of pediatric TB. MacLean et al. conducted a systematic review and meta-analysis for the detection of Mtb using fecal specimens and Xpert MTB/RIF and reported 67% (95% confidence interval [CI], 52–79%) and 99% (95% CI, 98–99%) sensitivity and specificity, respectively [20]. Kabir et al. conducted a study on Xpert MTB/RIF Ultra for the detection of TB using fecal specimens and concluded that Xpert MTB/RIF Ultra is more sensitive than the conventional Xpert MTB/RIF (58.6% vs. 37.9%, respectively), but the specificity was relatively lower (89.7% vs. 100%, respectively) [21] Fecal samples are a potential alternative specimen for the diagnosis of pulmonary TB, especially among children.

3 Pretreatment Methods

Sputum is a major clinical specimen for the diagnosis of TB because almost 80–90% of TB cases form pulmonary disease. Sputum specimens always pass through the oral cavity; therefore, contamination with general bacteria and/or fungi is common. If the specimen is directly inoculated on or into the culture medium, the contaminants grow earlier than the mycobacteria, and the culture fails. Therefore, it is necessary to inactivate microorganisms other than mycobacteria. In general, the pretreatment method consists of two steps: decontamination and concentration.

3.1 Sodium Hydroxide

Sodium Hydroxide (NaOH) has been used to liquefy and decontaminate sputum for several decades. It is very effective in reducing the contamination rate to 2–5% in solid and 5–10% in liquid media, and the very low cost makes it preferable for many laboratories. With regard to the use of NaOH, the original Petroff's method used 4% (some mention 8%) NaOH for decontamination followed by centrifugation and neutralization with HCl solution. It was a complicated method and improved to using modified method for simplification [22]. The modified Petroff's method is commonly used for simple culture with acidified egg-based culture medium. The process is as follows: first, the same volume of sputum and 4% NaOH solution is mixed by vortexing, and the mixture is left for 15 min at ambient temperature; second, the mixture is inoculated onto acidified medium directly so that the medium is neutralized by the remaining alkaline. The advantage of this modified Petroff's method is its simplicity, but 4% NaOH (2% even after mixing) can still kill more

than 40% of the Mtb in the specimen [23]. Another disadvantage is that there is no concentration of specimen, and thus, the culture sensitivity is limited. With the development of laboratory capacity, many laboratories have used centrifugation. In parallel, liquid culture, which is more sensitive than solid culture in general, has become available in many laboratories. However, the liquid culture medium Middlebrook 7H9, which is the most commonly used medium, has a lower buffering capacity than that of the solid medium, in which the buffer is the major component. To neutralize the high-pH specimen with NaOH efficiently, it is necessary to reduce the concentration of NaOH again. However, low NaOH (2% in original solution and 1% after mixture) is not sufficient to efficiently liquefy purulent sputum; therefore, N-acetyl-*L*-cysteine (NALC) is utilized. NALC is a precursor of the antioxidant glutathione and is commonly used to liquefy thick purulent sputum by cutting the disulfide bonds of heavily cross-linked mucus glycoproteins [24]. NALC-NaOH (2%) is currently the standard pretreatment solution for decontamination and liquefaction of sputum, typically combined with the concentration of specimen using centrifugation. However, although the NALC-NaOH method is simple and easy, it involves critical problems owing to the inactivation of Mtb. Thus, it is critical to maintain a standard operational procedure when pretreating sputum specimens with NALC-NaOH.

3.2 Chlorhexidine

Chlorhexidine is a potential decontamination material for Mtb culture. Chlorhexidine is a commonly used disinfectant and is even used for preparing culture agar plates for the recovery of environmental NTM, especially rapidly growing ones [25]. Asmar et al. developed a chlorhexidine agar plate culture medium protocol for the recovery of Mtb and compared its performance with that of the NALC-NaOH-Bactec MGIT (Becton Dickinson, Sparks, MD) protocol [26]. The respiratory specimen was decontaminated using 0.7% chlorhexidine and inoculated onto MOD9 culture medium. Briefly, equal volumes of 0.1% dithiothreitol and sputum specimens were mixed for 10 min in a 50 mL conical tube. Then, a triple volume of chlorhexidine solution was added. The mixture was vortexed and incubated for 15 min at room temperature with continuous agitation. The results indicated 96% sensitivity in chlorhexidine-MOD9 plates and 70% sensitivity in NALC-NaOH-Bactec MGIT ($p < 0.05$) among 50 positive results from 300 clinical specimens. Therefore, chlorhexidine can be a potential alternative to NALC-NaOH.

3.3 Concentration Methods

For better recovery of Mtb from clinical specimens, the concentration of Mtb should be as high as possible. Because the inoculum size for culture is fixed in many commercially available culturing devices (typically 500 μL), the fixed amount of sample

should contain as many Mtb as possible to obtain a successful culture result. Centrifugation is a commonly used concentration technique for clinical specimens. According to the Public Health Mycobacteriology: A Guide for the Level III Laboratory and other reports, centrifugation is recommended at 3000 × *g* for 15–20 min at 4 °C. Approximately 95% Mtb can be recovered from the NALC-NaOH-treated mixture under these conditions [27]. However, Ratnam et al. evaluated the effectiveness of relative centrifugal force (RCF) and time using clinical sputum specimens and concluded that with a 15–20 min centrifugation time, on average, 67–71% of mycobacteria were recovered at an RCF of 2074 × *g* and 76–80% were recovered at 3005 or 3895 × *g* at the maximum radius [28]. Yoshimatsu et al. simulated sputum specimens with Mtb suspension and THP-1 cells and tested the recovery of Mtb with and without THP-1 cells at different RCFs. They concluded that the specimens containing a higher number of bacteria and THP-1 cells tended to yield a higher concentration and recovery rate of Mtb ($p = 0.001$–0.083). In their study, Mtb was recovered more efficiently with THP-1 cells than without them, and 24.7–54.4% of Mtb was recovered with THP-1 cells through centrifugation at 3000 × *g* for 15 min. This study indicates that THP-1 can be a carrier of Mtb for efficient sedimentation [29]. Because Mtb has many lipids in the cell wall, it floats on the surface of the buffered sputum-NALC-NaOH mixture. For better concentration and sedimentation through centrifugation, Mtb requires carrier cells. In this regard, it is evident that obtaining purulent sputum specimens is quite important for highly sensitive detection of Mtb, indicating a sensitive bacteriological diagnosis of TB. The relative importance of centrifugation does not change even after these studies, but a more efficient concentration technique is required. Unfortunately, this centrifugation problem has not been resolved to date.

3.4 Culture Methods

Culture technologies, including liquid and solid media, are still fundamental for the bacteriological confirmation of TB. The purposes of mycobacterial culture are (1) high sensitivity detection of Mtb and (2) the recovery of live Mtb for further examinations, including phenotypic drug susceptibility testing (DST), follow-up of TB treatment, and next-generation whole-genome sequencing. The liquid culture maintains the highest sensitivity for the detection of Mtb from clinical specimens, and automated systems are the major players. The liquid culture is relatively susceptible to contamination, and only fresh specimens can be used for liquid culture. The LoD of the liquid culture is considered to be 10–100 CFU/mL.

As a new culture technique, the MOD9 protocol was reported by Asmar et al. MOD9 solid agar medium comprises 5% sheep blood, 15% heat-inactivated lamb serum, and 100 mg/L ascorbic acid. A mixture of azorubine and Ponceau 4R, which are used as food dyes, is added to stain the medium red. The authors reported that the turn-around time (TAT) of MOD9 when inoculated with 10 CFU of Mtb (100 μL of 100 CFU/mL Mtb suspension) was 4.9 ± 1 days, whereas that of the

Löwensterin-Jensen (L-J) medium was 10.8 ± 1.7 days [30]. As described in the previous paragraph, chlorhexidine-MOD9 culture is superior to NALC-NaOH-Bactec MGIT, therefore, it could be an alternative culture method for the L-J medium. In another study by the same group, microaerophilic conditions accelerated the growth of Mtb. The authors reported an approximately 40% decrease in TAT under microaerophilic conditions [31].

4 Nucleic Acid Amplification

Nucleic Acid Amplification (NAA) is a powerful tool for detecting Mtb, specifically with a relatively high sensitivity and short TAT. NAA was developed in 1983 by Dr. Kary Mullis and termed as polymerase chain reaction (PCR) [32]. It utilizes oligonucleotides and DNA polymerase and repeats DNA synthesis in vitro. PCR can amplify one double-stranded DNA to millions in approximately 2 h, and the primer design can amplify a specific target so that Mtb detection and identification can be performed simultaneously. Many different DNA/RNA amplification methods have been developed after the invention of PCR. NAA was performed manually two decades ago, but the amplification and detection/identification processes were integrated into one process, and the TAT of the current NAA is very short (less than 1 h). In addition, the original PCR is a qualitative method, but real-time monitoring technology makes NAA semiquantitative. Moreover, many recent molecular technologies are based on the NAA.

However, NAA has a limitation in that it cannot be used for the follow-up of TB treatment. NAA can detect any target DNA, regardless of the viability of Mtb bacilli. Moore et al. reported long-lasting NAA positive results using transcription-mediated amplification after successful TB treatment compared with the smear and culture examinations [33]. This is because the target was rRNA. Viable and dead Mtb can be potentially differentiated by detecting mRNA. Therefore, it is not recommended to use NAA for the follow-up of TB treatment [34].

4.1 Xpert Series

The Xpert series is a world-famous automated NAA system that detects Mtb and rifampicin (RIF) resistance by detecting genetic mutations. Xpert MTB/RIF (Cepheid, Sunnyvale, CA) was the first product used to detect Mtb and RIF resistance with the GeneXpert (Cepheid, Sunnyvale, CA) device, followed by Xpert MTB/RIF Ultra and XDR. The first device was released in 2010 [35]. The platform of the Xpert series is same; a multichambered cartridge is driven by the air pressure generated by a plunger in the center. The microfluidic system driven by the valve drive automatically proceeds with DNA extraction, purification, real-time amplification, and detection processes. The multiple chambers contain different reagents/

solutions, and the target DNA moves along the chambers in a programmed order. The real-time PCR products are scanned with a molecular beacon, and Mtb and RIF resistances are detected [36]. The results are analyzed using exclusive computer software and indicated on the monitor automatically.

4.1.1 Xpert MTB/RIF

Xpert MTB/RIF is the first rapid Mtb test recommended by WHO in December 2010 (G3), and it is still used mainly as a frontline test in many countries with a high TB burden (G4). According to the Cochrane Xpert MTB/RIF review in 2013, the pooled sensitivity is 98% (95% CI, 97–99%) for smear-positive/culture-positive TB and 68% (95% CI, 59–75%) for smear-negative/culture-positive pulmonary TB. Among people living with HIV (PLHIV), the pooled sensitivity is 80% (95% CI, 67–88%). For rifampicin resistance detection, the pooled sensitivity is 94% (95% CI, 87–97%) and the pooled specificity is 98% (95% CI, 97–99%) [37]. A new Cochrane review of Xpert MTB/RIF in 2019 reported a pooled sensitivity and specificity of 98% (95% CI, 97–98%) in smear-positive/culture-positive samples and 67% (95% CI, 62–72%) in smear-negative/culture-positive pulmonary TB samples, respectively [38]. However, Sohn et al. reported that when the Xpert MTB/RIF assay was performed in a low TB incidence setting in Montreal, Canada, it showed a sensitivity of 46% (95% CI, 26–67%) and a specificity of 100% (95% CI, 99–100%) for the detection of Mtb [39]. Tsuyuguchi et al. also reported that the Xpert MTB/RIF in Japan (as of 2015) among Mtb culture-positive specimens showed a positivity of 95.2% (95% CI, 91.2–97.5%) in smear-positive specimens and 44.7% (95% CI, 30.1–60.3%) in smear-negative specimens [40]. For RIF resistance detection, the prevalence of RIF varies in different areas, and thus, the sensitivity also differs [41]. These data indicate that the performance of Xpert MTB/RIF may depend on the TB incidence and prerequisite probability, similar to that of other laboratory tests.

Prerequisite probability directly affects the predictive values in clinical practice. In many countries with a high TB burden, the prerequisite probability of TB at clinics/hospitals is expected to be relatively high, and the use of any laboratory examinations is reasonable. The following formula is used to calculate the predictive values (Table 4.1).

With an NAA of 94% sensitivity and 98% specificity, the positive predictive value (PPV) will be 32.2% if true positivity is only 1% (Fig. 4.1). However, if the true positivity is 10%, PPV will be 83.9%. Therefore, before using NAA, the prerequisite probability of TB in the target population/individual should be relatively high. Otherwise, the positive NAA result is not significant. In other words, NAA should not be used for people without presumed TB, indicating that it cannot be used for the first screening. This is also applicable for the detection of RIF resistance mutations using Xpert MTB/RIF.

Table 4.1 Calculation of sensitivity, specificity, positive predictive value, negative predictive value, and agreement

	Standard/Positive	Standard/Negative	Total
Test result/positive	a	b	e
Test result/negative	c	d	f
Total	g	h	i

Sensitivity; $a/(a + c)$
Specificity; $d/(b + d)$
Positive predictive value; $d/(b + d) \times (1 - R)/\{d/(b + d) \times (1 - R) + (1 - a/(a + c)) \times R\}$
Negative predictive value; $a/(a + c) \times R/\{a/(a + c) \times R + (1 - d/(b + d)) \times (1 - R)\}$
Agreement; $(a + d)/(a + b + c + d)$
R; True positivity among the target population

Prevalence	Positive Predictive Value	Negative Predictive Value
0.001	0.044933078	0.999938718
0.002	0.086080586	0.999877321
0.005	0.191056911	0.999692434
0.007	0.248865356	0.999568594
0.01	0.321917808	0.999381953
0.02	0.489583333	0.99875208
0.03	0.592436975	0.998110038
0.04	0.661971831	0.997455471
0.05	0.712121212	0.996788009
0.06	0.75	0.996107266
0.07	0.779620853	0.995412844
0.08	0.803418803	0.994704325
0.09	0.822957198	0.993981275
0.1	0.839285714	0.993243243
0.11	0.853135314	0.992489759
0.12	0.865030675	0.991720331
0.13	0.875358166	0.990934449
0.14	0.884408602	0.990131579
0.15	0.892405063	0.989311164
0.16	0.899521531	0.988472622
0.17	0.905895692	0.987615347
0.18	0.911637931	0.986738703
0.19	0.916837782	0.985842027
0.2	0.921568627	0.984924623

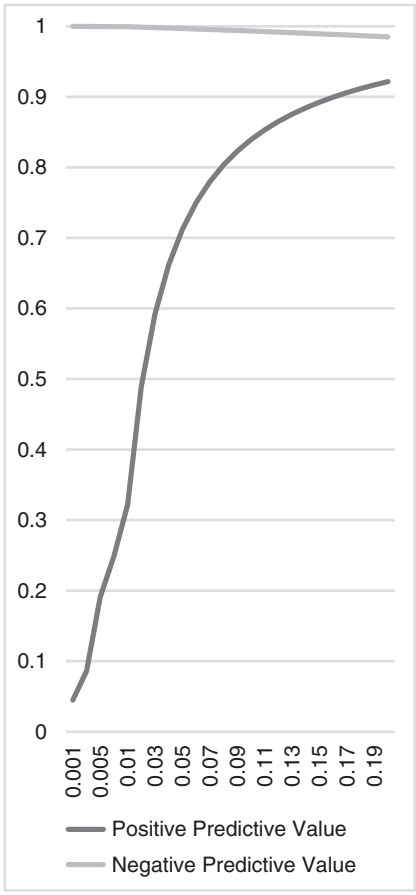

Fig. 4.1 Positive and negative predictive values on various prerequisite probability (prevalence)

Given the condition for clinical NAA use above, NAA is generally used in presumed patients with TB who have a high possibility of active disease and in relapsed TB cases with a high probability of drug resistance [42].

Programmatic management of drug-resistant TB (PMDT) is adopted in many countries where TB is still prevalent [43, 44]. PMDT is an algorithm used to detect multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB). The program uses Xpert MTB/RIF as a first screening examination because the target patients are relapsed cases with a high possibility of RIF resistance. If the first Xpert MTB/RIF screening is positive (RIF resistant), the patient is considered to have MDR-TB. In this program, RIF resistance is used as a surrogate for MDR-TB because RIF resistance is accompanied by isoniazid (INH) resistance with a high probability [45, 46]. However, it varies depending on the prevalence of RIF monoresistance [47]. Confirmation of MDR-TB may be required in settings where the prevalence of RIF resistance is low when Xpert MTB/RIF is positive for RIF.

4.1.2 Xpert MTB/RIF Ultra

Xpert MTB/RIF Ultra (Cepheid, Sunnyvale, CA) is a second-generation cartridge that can detect Mtb and RIF resistance similar to Xpert MTB/RIF but with higher sensitivity and specificity. The LoD of Mtb using the conventional Xpert MTB/RIF is 131 CFU/mL, according to the manufacturer's instructions. For the mutation detection of *rpoB*, which is responsible for RIF resistance, the conventional Xpert MTB/RIF detects synonymous mutations without amino acid substitution (silent mutation), [48] and generates false-positive results in specific sequences. The LoD of Xpert MTB/RIF Ultra becomes 16 CFU/mL by incorporating increased amplification reaction volume (from 25 μL to 50 μL), new Mtb detection targets (IS*6110* and IS*1081*), and new DNA polymerase. The LoD of the Xpert MTB/RIF Ultra was calculated using Mtb H37Rv, which contains 16 copies of IS*6110* and 5 copies of IS*1081*. The LoD of *M. tuberculosis* var. BCG is 143.4 CFU/mL [49]. The problem of detecting silent mutations is solved using melting curve analysis after amplification and hybridization of the target gene and molecular beacons (probes for wild-type sequence). These modifications increase the sensitivity and sequence specificity.

In March 2017, the WHO released the meeting report of a technical expert consultation and recommended the use of Xpert MTB/RIF Ultra cartridge as a replacement for the conventional Xpert MTB/RIF [50, 51]. In the meeting report, it is indicated that the overall sensitivity of Xpert MTB/RIF Ultra for detecting Mtb is 5% (95% CI, 2.7–7.8%) compared with that of Xpert MTB/RIF, whereas the specificity decreased by 3.2% (95% CI, 2.1–4.7%). The sensitivity increased by 17% (95% CI, 10–25%) and 12% (95% CI, 4.9–21%) among smear-negative/culture-positive patients and patients with TB living with HIV, respectively. The WHO provided a statement as follows: "the Ultra assay is non-inferior to the current Xpert MTB/RIF assay for the diagnosis of MTB and the detection of rifampicin resistance and can be used as an alternative to the latter in all settings." The Xpert MTB/RIF Ultra cartridge can function in a conventional GeneXpert device, but software

updates are required. The TAT of the Xpert MTB/RIF Ultra test reduced to 50 min from 110 min in the conventional Xpert MTB/RIF.

However, the increased sensitivity of the Xpert MTB/RIF Ultra has resulted in some clinical problems. Owing to the semiquantitative nature of the Xpert series, Xpert MTB/RIF Ultra shows Mtb detection results as trace, very low, low, moderate, and high. The trace result indicates that one or both of the probes for the multicopy targets are positive with Ct values less than 37 cycles and not more than one *rpoB* probe has a Ct value of less than 40 cycles. This also means that Mtb is detected and RIF is indeterminate in the trace results. The WHO technical expert group concluded that trace positivity is a sign of initiating anti-TB treatment in PLHIV, children, and patients with extrapulmonary TB. However, for patients with pulmonary TB without HIV, the expert group recommends repeating the Xpert MTB/RIF Ultra test when trace results are obtained in the initial test.

In 2020, the WHO released new consolidated guidelines for the intended use of molecular tests and provided the following recommendations [12]. In the guideline, WHO recommends on Xpert MTB/RIF and Xpert MTB/RIF Ultra (Xpert Ultra) as initial tests in adults and children with signs and symptoms of pulmonary and extrapulmonary TB. WHO also makes recommendations for the repeated use of Xpert MTB/RIF and Xpert Ultra with signs and symptoms of pulmonary TB.

4.1.3 Xpert MTB/XDR

Xpert MTB/XDR is a newly developed Xpert series cartridge that detects INH resistance using the *inhA* promoter, *katG*, f*abG1*, *oxyR-aphC* intergenic region, ethionamide using the *inhA* promoter, fluoroquinolones (FQs) using *gyrA* and *gyrB*, and second-line injectables (amikacin [AMK], kanamycin [KM], and capreomycin [CPM]) using *rrs* and *eis* [52]. This new cartridge requires a new module to perform the test to read 10 colors using multiple probes. Cao et al. reported concordance between phenotypic DST (pDST) and genotypic DST (gDST), with a sensitivity of 65.4–98.3% and 88.5–100% and specificity of 95.0–99.7% and 97.3–100%, respectively [53]. However, INH sensitivity varies in different settings; therefore, validation may be necessary when a country introduces Xpert MTB/XDR.

4.2 TB-Loop-Mediated Isothermal Amplification (TB-LAMP)

LAMP, a type of NAA, was developed by Notomi et al. in 2000 [54]. This amplification technology uses six different sequences for priming amplification, and the reaction proceeds under isothermal conditions. Then, a simple heating device is required for amplification. The amplification result is observed using the turbidity of the metabolite and fluorescence, and the result is semiquantitative and qualitative (end-point evaluation) [55].

The WHO recommended TB-LAMP (Eiken Chemical Co., Ltd., Tokyo, Japan) in August 2016 and released its policy guidance [56]. TB-LAMP is a fully manual NAA with an 11-step procedure. The WHO recommends the use of TB-LAMP as a replacement test and for follow-up of smear microscopy for the diagnosis of pulmonary TB in adults. Shete et al. reported that its pooled sensitivity was 77.7% (95% CI, 71.2–83.0%) and specificity was 98.1% (95% CI, 95.7–99.2%). They also reported differences in the performance of TB-LAMP and Xpert MTB/RIF for the detection of Mtb; the pooled sensitivity difference was −2.5% (95% CI, −8.0–2.9%) and the pooled specificity difference was −1.8% (95% CI, −0.9–1.8%). In clinical practice, Yadav et al. reported that in 218 cases, the sensitivity of Xpert MTB/RIF and TB-LAMP, when the culture was taken as a reference standard, was 90% (95% CI, 78.2–96.7%) and 82% (95% CI, 68.6–91.4%), respectively. The specificity, PPV, and negative predictive value of the TB-LAMP assay were 96.8% (95% CI, 92.8–98.9%), 89.1% (95% CI, 77.4–95.2%), and 94.4% (95% CI, 90.4–96.5%), respectively [57]. At a peripheral health center level in Uganda, with 233 presumptive patients with TB including 113 PLHIV, TB-LAMP showed a sensitivity and specificity of 55.4% (95% CI, 44.1–66.3%) and 98.0% (95% CI, 94.3–99.6%), respectively. In the same study, the sensitivity of smear microscopy was 18.5%. Among the smear negatives, TB-LAMP sensitivity and specificity were 24.4% (95% CI, 12.9–39.5%) and 98.6% (95% CI, 95.1–99.8%), respectively [58]. In addition, the data of a study conducted in Malawi were unique. Of 53 bacteriologically confirmed TB samples, the sensitivity of TB-LAMP was 65.0% (95% CI, 48.3–79.4%) with 100% (95% CI, 98.0–100%) specificity, whereas the sensitivity of Xpert MTB/RIF was 77.5% (95% CI, 61.5–89.2%) ($p = 0.132$). Interestingly, the sensitivity of concentrated fluorescent smear microscopy was 87.5% (95% CI, 73.2–95.8%), which was higher than that of TB-LAMP and Xpert MTB/RIF [59].

4.3 TrueNAT

Truenat™ MTB and Truenat™ MTB-RIF Dx (Molbio, Verna, India) are also types of NAAs that were endorsed by the WHO in July 2020 [60]. Truenat is a chip-based, battery-operated, real-time PCR kit. It uses the Trueprep AUTO Universal Cartridge-Based Sample Prep kit (Molbio, Verna, India) for the preparation of DNA from pretreated specimens and the Truelab™ Real-Time micro PCR Analyzer (Molbio, Verna, India) for real-time PCR and automated judgment of the results. The Truenat™ MTB chip and Truenat™ MTB-RIF Dx chip are also employed in the Truelab™ Real-Time micro PCR Analyzer. The TrueNAT chip is a type of micro-total analysis system. The WHO rapid communication in January 2020 indicates that the sensitivity and specificity of the Truenat MTB assay are 83% and 99%, respectively. Nikam et al. reported that the sensitivity of the Truenat MTB assay was 98.9% (95% CI, 94.2–100%) in smear-positive/culture-positive specimens and 86.2% (95% CI, 74.1–93.4%) in smear-negative/culture/positive specimens. The specificity of the Truenat MTB assay was 52.9% (95% CI, 43.6–61.9%) in the study [61].

Georghiou et al. reported the performance of Truenat™ MTB-RIF Dx. Only 19 specimens were tested, and the sensitivity of detecting RIF resistance mutations was 98.6% (95% CI, 90.5–91.8%). They concluded that Truenat™ MTB-RIF Dx shows similar performance to that of GenoType® MTBDR*plus* (Bruker-Hain Lifescience, Germany) [62]. More studies are required to obtain stringent data on these newly endorsed kits.

4.4 Line Probe Assay (LPA)

The reverse hybridization LPA is a commonly used probe hybridization technology for detecting wild type and/or mutated sequences normally on nitrocellulose strips. Wild type and/or mutated sequence probes are immobilized on the strip, and the target is amplified using PCR with biotinylated primers, which hybridize with a specific probe. The hybrid is detected by the avidin–biotin complex.

GenoType® MTBDR*plus* and *sl* are the products of Bruker-Hain Lifescience (Germany) and are recommended by the WHO for the detection of anti-TB drug resistance [63, 64]. MTBDR*plus* can detect INH and RIF resistance by detecting mutations in the *inhA* promoter, *katG*, and *rpoB*. Similarly, MTBDR*sl* can detect FQs, aminoglycosides, and ethambutol (EMB) by detecting mutations in *gyrA*, *rrs*, and *embB*. These kits are commonly used in PMDT to identify extensive drug-resistant Mtb (old definition: WHO updated the definition of XDR-TB in 2021) [65].

Bai et al. conducted a meta-analysis on GenoType® MTBDR*plus* and reported a pooled sensitivity of 91% (95% CI, 88–94%) for INH and 96% (95% CI, 95–97%) for RIF. The specificity was 99% (95% CI, 98–99%) for INH and 98% (95% CI, 97–99%) for RIF [66]. Tomasicchio M et al. reported the sensitivity for INH and RIF are 95.4% and 89%, respectively (MTBDR*plus* ver. 1.0), in sputum specimens. They also reported the specificity for INH and RIF are 97.7% and 91.8%, respectively (MTBDR*plus* ver. 1.0) [67]. Seifert M et al. investigate the performance of MTBDR*plus* (ver. 2.0) in smear-positive and negative specimens separately. The sensitivity and specificity of INH in smear-positive specimens were 94.9% (95% CI, 92.4–96.6%), 100% (95% CI, 97.9–100%), respectively, and in and negative were 81.6% (95% CI, 65.1–91.7%), and 98.1% (95% CI, 88.6–99.9%), respectively. Similarly, the sensitivity and specificity of RIF in smear-positive specimens were 97.1% (95% CI, 94.9–98.4%), 98.5% (95% CI, 96.0–99.5%), respectively, and in and negative were 91.4% (95% CI, 75.8–97.8%), and 95.2% (95% CI, 85.6–98.7%), respectively [62].

Tomasicchio et al. also evaluated the performance of MTBDR*sl* (ver. 1.0) and reported that the sensitivity and specificity of ofloxacin (OFX, FQ) were 58.9% and 61.6%, respectively. Similarly, they reported sensitivity and specificity of AMK were 100% and 100%, respectively [67]. Gardee et al. reported the performance of the MTBDR*sl* (ver. 2.0) in South Africa; the sensitivity and specificity of FQ (OFX) were 100% (95% CI, 95.8–100%) and 98.9% (95% CI, 96.1–99.9%); second-line injectables (AMK, KM, and CPM) were 89.2% (95% CI, 79.1–95.6) and 98.5% (95% CI, 95.7–99.7%) [68].

Genoscolor PZA-TB II (Nipro Co., Osaka, Japan) is used to detect *pncA* and its promotor mutations/indels. Willby et al. reported that the sensitivity and specificity of the kit for detecting pyrazinamide (PZA) resistance were 93.2% (95% CI, 89.3–95.8%) and 91.2% (95% CI, 77.0%–97.0%), respectively [69]. Mitarai et al. reported that the sensitivity and specificity of Genoscolor PZA-TB (Nipro Co., Osaka, Japan) were 89.7% and 96.0%, respectively [55]. Aono et al. reported large deletions in three Mtb strains around *pncA* (1565 bp, 4475 bp, and 6258 bp) showing high PZA resistance [70]. Because pDST of PZA using MGIT PZA AST (Becton Dickinson, Sparks, MD) has been reported to show relatively high false resistance, the pyrazinamidase test is commonly used as an alternative. Aono et al. also reported a simple pyrazinamidase test procedure using a liquid culture medium [71].

5 Species Identification

The identification of *Mycobacterium* species is critical in many settings. In resource-limited settings, Mtb identification is relatively easy, but NTM identification is not. In general, national reference laboratories have the capacity to perform LPA for the identification of major species [72], but rare species are generally neglected (*Mycobacterium* spp.). In industrialized countries, the incidence of NTM infections is increasing based on the decrease in TB incidence (trade-off), and the isolation of even rare species is common. General LPA does not cover rare NTM species, and sequencing of multilocus housekeeping genes can be used to identify rare species [73, 74]. Matrix-assisted laser deionization (MALDI)–time of flight mass spectrometry (TOF MS) is now commonly used in many industrialized countries because it is a simple, easy, and rapid process [75].

5.1 MPT64

MPT64 is a secretory antigen that is specific to Mtb. The antigen is used for the identification of Mtb. Capilia TB or TB-Neo (TAUNS, Izunokuni-shi, Japan) is a lateral flow immunoassay to identify cultured Mtb [76]. Its LoD is approximately 10^5 CFU/mL; therefore, the direct detection of Mtb from clinical specimens is difficult. Chikamatsu et al. reported the specificity of Capilia TB-Neo using 96 mycobacterial species (4 Mtb and 92 NTM) and showed 100% agreement with a subset of the strains tested. They also evaluated 500 Mtb (including three Mtb BCG) and confirmed a sensitivity of 99.6%. Two Mtb isolates with Capilia TB-Neo-negative results had a point mutation and 63-bases deletion in the *mpt64* gene [77]. These mutations are the most common cause of Capilia TB negative results [78]. The SD Bioline TB Ag MPT64 Rapid test (Abott) and BD MGIT TBc Identification Test (TBc ID, Becton Dickinson) showed similar performance for Mtb identification. [79, 80].

5.2 *Matrix-Assisted Laser Deionization–Time of Flight Mass Spectrometry (MALDI-TOF MS)*

MALDI-TOF MS is a combination of MALDI and TOF MS. In mass spectrometry, molecules are converted into gaseous ions by some method and move in a vacuum by electromagnetic force, and these ions are separated according to their mass-to-charge ratios, which causes differences in flight times. Laser irradiation is often used as the ionization method, and MALDI is often used. Recently, a method of using an ionization supporting base instead of a matrix has been reported. By combining this with TOF MS, which is a separation method based on the mass-to-charge ratio of ions, MALDI-TOF MS is established.

The principle of MALDI-TOF MS is based on the following: the protein extracted from the target to be analyzed (acid-fast bacillus in this case) is put into a matrix (α-cyano-4-hydroxysilicate cinnamic acid in this case) and organic solvent (a mixture of 250 μL of acetonitrile 50%, ultrapure water 47.5%, and trifluoroacetic acid 2.5%) and mixed. The main component of the protein sample is ribosomal protein. When a uniformly mixed matrix is placed on a substrate and irradiated with a nitrogen laser, the matrix absorbs the laser and converts it into thermal energy. Then, a part of the mixture of the matrix and sample is instantly heated and vaporized. At this time, cations of various sizes are generated on the sample substrate. The sample substrate, with a potential of zero, is placed in a vacuum with an electric field, and the grid electrodes are installed above the vacuum space. Therefore, the cations are accelerated upward from the base position toward the grid electrode. Because the potential difference between cations of various sizes is constant (the kinetic energy is the same), the lighter the ion, the faster it reaches the detector, and the heavier the ion, the slower it reaches the detector. It is possible to obtain the type of weight as a spectrum and the intensity (amount) of ions detected by the detector. The obtained mass spectrum is strain specific, and it is possible to identify the strain by comparing it with the reference strain data and the clinical isolates collected in the database.

Currently, the clinically used MALDI-TOF MS acid-fast bacilli identification devices include Bruker Daltonics' Biotyper and BioMérieux's Vitec MS. According to the Bruker Daltonics website (https://www.bruker.com/en/products-and-solutions/microbiology-and-diagnostics/microbial-identification/mbt-mycobacteria-ruo.html), 178 acid-fast bacilli can be identified using the current database ver. 6.0. Similarly, Vitec MS can identify 37 species and two groups of acid-fast bacilli (https://www.biomerieux.co.uk/product/vitekr-ms). In any case, the accuracy of identification depends on the richness of the database; therefore, it is recommended to update it as soon as a new mass spectrum database is released.

5.3 Multilocus Sequencing Typing (MLST)

MLST normally uses 16S rRNA, *hsp65* [81], and *rpoB* [82]. However, the sequences of limited number of housekeeping genes are not enough to identify *Mycobacterial* species (internal data). Nakamura et al. recently developed an MLST procedure using 184 genes from the whole-genome sequence (MinION, Oxford Nanopore, UK) and identified a total of 175 NTM species [83]. MLST requires the sequencing capacity of multiple genes; therefore, it is not easily performed as a general procedure.

6 Biomarkers

There are two types of biomarkers: bacteria and host. In this section, the bacterial biomarkers for the diagnosis and treatment follow-up of TB are described.

6.1 ESAT-6 and CFP-10

ESAT-6 and CFP-10 are Mtb-specific secretory antigens located in RD1 [84]. These antigens circulate in the blood of patients with active TB and are used for the diagnosis of active TB. They are identified by direct TB antigen detection in the blood. Liu et al. reported a method for collecting and detecting secreted specific proteins from tubercle bacilli. They collected and concentrated ESAT-6 and CFP-10 using Nanodisk conjugated with specific antibodies and detected these antigens using MALDI-TOF MS. [85] In this case, the Nanodisk refers to the porous discoidal silicon nanoparticles. Porous silicon was used as the nanovector. In this study, the LoDs of ESAT-6 and CFP-10 were 200 pM and 50 pM, respectively. When tested in individuals (HIV negative), the sensitivity and specificity were 92.6% and 100%, respectively. Liu et al. conducted a clinical evaluation of 376 patients and reported a sensitivity of 88.3% and specificity of 95.8% [86].

6.2 Lipoarabinomannan

To cope with the above-mentioned problems of culture examination, several biomarkers have recently been developed. As one diagnostic method that uses urine specimen, Alere determine TB-LAM Ag (Abbott, US) is already endorsed by WHO and is in clinical use for the diagnosis of TB in PLHIV with less than 200/μL CD4 cells, exhibiting 39.0–66.7% sensitivity [87]. This technology detects lipoarabinomannan (LAM), a core cell wall component of mycobacteria, in urine specimens. LAM antigen commonly exists in the genus *Mycobacterium*; therefore, it is not specific to *M. tuberculosis* but can be used for the diagnosis and follow-up of highly

presumed TB cases. Recently, Broger et al. have reported that a new urine LAM detection kit named SILVAM TB-LAM (Fujifilm, Japan) increased the sensitivity by approximately 25–30% compared with that of Alere Determine TB-LAM Ag, resulting in 43.9–87.1% detection in patients with pulmonary and extrapulmonary TB [88, 89]. Thus, as a bacteriological biomarker that does not require culture examination, it is proven to be a diagnostic tool for TB by detecting cellular LAM components. Kawasaki et al. recently reported the use of the same LAM antigen. They used the LAM antigen as a biomarker for the follow-up of treatment effects. Although the mechanisms of quick clearance of cellular components are unknown, the amount of LAM antigen in the sputum reflects the live *M. tuberculosis* bacteria, showing a clear correlation [90]. It showed a quick reduction (within 1 week) of LAM antigen after effective anti-TB treatment. The authors suggested the use of LAM as a biomarker for the observation of treatment outcomes. It can be useful for the follow-up of anti-TB treatment without a culture examination.

6.3 Immunogenic Protein MPT64

Recently, Sakashita et al. reported similar but different culture-free diagnostic and follow-up systems. They evaluated MPT64, which is an Mtb complex (MTC)-specific secretory antigen, for the diagnosis and follow-up of patients with TB [91]. MPT64 is a well-known antigen excreted through the ESX system of *M. tuberculosis* and is already used in several commercial MTC identification kits, such as Capilia TB-Neo (Tauns, Japan) [77] and SD BIOLINE TB Ag MPT64 rapid (Standard Diagnostics, Korea) [79]. Therefore, the species specificity of MPT64 is quite high, reaching almost 100%. MPT64 is a secretory antigen; therefore, its secretion can assure the viability of MTC directly. Owing to these advantages, species specificity, and viability indication, it is useful for the diagnosis of MTC infection in active TB and for the follow-up of anti-TB treatment effects without culture examination. The authors reported a similar diagnostic capacity of the system with conventional liquid culture examination and showed a clear decline in MPT64 levels after effective anti-TB treatment. The same system is used for the diagnosis and follow-up of TB treatment. It is possible to replace culture examinations with this system.

7 Drug Susceptibility Testing

7.1 Phenotypic DST

Drug susceptibility testing is a method for reproducing and estimating the in vivo drug–pathogen–living body interaction and the resulting “clinical effect” in vitro. Therefore, “drug dose,” “bacterial parameters such as MIC (minimum inhibitory concentration),” “biological parameters such as drug concentration in blood/tissue,”

and "immunity involvement," should be clinically evaluated and the drug effect should be estimated. A susceptibility test was established only when the in vitro association of the pathogen is shown to correlate with the clinical effect. Specifically, even if a certain standard value is set by the MIC, unless it is clinically clearly stated that the clinical effect of the drug is different (effective or not) from the standard value, the reference value has no meaning. The same is true for Mtb, and its accuracy varies depending on the drug [92]. Currently, the recognized (or virtually used) anti-TB drugs are INH, RIF, rifabutin (RBT), pyrazinamide (PZA), streptomycin (SM), EMB, AMK, KM, CPM, ETH, cycloserine (CS), Para-aminosalicylic acid (PAS), and FQs, used generally in most countries. In addition, moxifloxacin (MFX), linezolid (LZD), clofazimine (CFZ), bedaquiline (BDQ), and delamanide (DLM) are also used, especially for M/XDR-TB bacteria. Of these, INH, RFP/RBT, EMB, SM, PZA, KM/AMK/CPM, and LFX/MFX. have relatively high accuracy (high sensitivity/specificity), but other drugs with low test accuracy have been reported [93].

pDST for Mtb has been almost established, including the absolute concentration method, resistance ratio method, proportion method, and minimum inhibitory concentration (MIC) method [94]. Currently, the proportion method is widely used in many countries because of its relatively easy procedure.

There are two types of media for pDST: solid and liquid. L-J is a common basic medium for pDST, but it does not adapt to new drugs, including BDQ, DLM, MFX, LZD, and CFZ. For the pDST of these drugs, the agar proportion and/or MGIT AST (Becton Dickinson, Sparks, MD) are available, mainly because of the available critical concentrations for these drugs (Table 4.2) [95]. Some (or many) of the pDST for these drugs should be performed using a drug-containing medium prepared in-house. Quality control measures should be strictly monitored. As another version of liquid culture-based pDST, MIC is available for several drugs. However, the currently proposed MIC breakpoints are not confident even in CLSI M62 (only for INH, RIF, and EMB) [96]. MIC takes 10–14 days to complete in general, but sometimes takes up to 21 days. In general, the TAT of pDSTs is not rapid. Currently, MGIT AST directly from a positive MGIT culture is as fast as pDST (TAT 2–4 weeks) [97, 98].

Microscopic observation drug susceptibility (MODS) assay is one of the relatively rapid pDSTs are available. This assay is a direct pDST using pretreated clinical specimens and a simulated proportion method in a 24-well microplate with a liquid medium containing antibacterial and fungal agents. For the detection of Mtb growth, MODS uses microscopy, therefore, the detection of Mtb growth is fast. The TAT of MODS is approximately 7 days after collection of clinical specimens, so it is faster compared to the indirect pDST, which uses culture Mtb. Moore et al. reported that the agreement of MODS with conventional reference pDST for INH and RIF was 97% and 100%, respectively [99]. Wikman-Jorgensen et al. conducted a systematic review and meta-analysis and reported the sensitivity and specificity for MDR-TB to be 89% (95% CI, 66.1–97%) and 100% (95% CI, 94.8–100%), respectively [100]. The user's guide is available from the Universidad Peruana Cayetano Heredia. A commercial kit TB MODS Kit™ (Hardy Diagnostics, Santa Maria, CA) is also available. MODS is reliable for INH and RIF resistance detection [101].

Table 4.2 Critical concentrations of anti-TB drugs (ref. [95])

Drug group	Drug	L-J	7 J10	7H11	MGIT
Group A	Levofloxacin (CC)	2.0	1.0	–	1.0
	Moxifloxacin (CC)	1.0	0.5	0.5	0.25
	Moxifloxacin (CB)	–	2.0	–	1.0
	Gatifloxacin (CC)	0.5	–	–	0.25
	Bedaquiline	–	–	0.25	1.0
	Linezolid	–	1.0	1.0	1.0
Group B	Clofazimine	–	–	–	1.0
	Cycloserine	–	–	–	–
	Terizidone	–	–	–	–
Group C	Ethambutol	2.0	5.0	7.5	5.0
	Delamanid	–	–	0.016	0.06
	Pyrazinamide	–	–	–	100
	Imipenem-cilastatin	–	–	–	–
	Meropenem	–	–	–	–
	Amikacin	30.0	2.0	–	1.0
	(or streptomycin)	4.0	2.0	2.0	1.0
	Ethionamide	40.0	5.0	10.0	5.0
	Prothionamide	40.0	–	–	2.5
	p-aminosalicylate	–	–	–	–

Another rapid pDST with a direct specimen is the thin-layer agar (TLA) assay. This is not a new method but also uses microscopy to observe growing colonies on thin-layer agar plates. Mejia et al. reported that the TAT of Mtb recovery from clinical specimens was 10 days for 60% and 14 days for 80% positives ($n = 761$) [102]. The colony morphology was distinctive, even under microscopy. Aldizzoni et al. reported that pDST with TLA detects RIF resistance with 93.0% sensitivity (95% CI, 77.4–98.0%) and 99.4% specificity (95% CI, 96.7–99.9%) within a median of 18.4 days [103].

7.2 Genetic DST

Genetic DST (gDST) is a drug resistance estimation method based on genetic mutations/indels that cause amino acid substitutions or translation errors in general. It is common to detect RIF resistance with gDST using Xpert MTB/RIF. It is also common to use LPA, such as MTBDR*plus* and *sl*, in PMDT in many countries as of today. Commercially available gDSTs have already been described in the subsection of the NAA (sub-section 5.4).

The WHO has released guidance for the clinical use of next-generation sequencing (NGS) to identify mutations/indels in respective Mtb genes at one time [104]. NGS can identify genome-wide mutations and indels, therefore, the sensitivity is relatively high compared to Xpert MTB/RIF and LPA [105].

The confidence of the gDST result will completely depend on the database used for the interpretation of the mutations and indels. The above-mentioned WHO guidance grades the confidence level of mutations/indels through a systematic review of published data. The most famous discussion of gDST confidence is RIF resistance. RIF binds to the beta-subunit of DNA-dependent RNA polymerase complex and inhibits the enzymatic capacity to kill Mtb [106]. The beta subunit is coded in the *rpoB* gene, and the mutations/indels in this gene cause a change in the affinity of RIF to the beta-subunit, resulting in resistance. There is a "hot spot" in *rpoB*, where many mutations/indels are accumulated in 81-bp, called the rifampicin resistance-determining region (RRDR) [107]. Even in this short region, many different mutations/indels are observed, and each mutation/indel may yield different MIC values. Some mutations are very confident with high MIC to RIF, but others raise MIC rather low [108]. These differences raise the issue of so-called "disputed mutation" with which the pDST and gDST may differ. Van Deun et al. reported this issue in 2013 and pointed out the discrepancies between gDST and pDST [109]. Thus, the confidence evaluation of any mutation/indel will be of great importance as far as gDST is concerned.

There are roughly two types of NGS: short read and long read. The short-read sequencing is well represented by the Illumina platform, while the long read is by Nanopore (Oxford Nanopore Technologies) and PacBio (Pacific Bioscience) technologies. The former is considered as second generation and the latter is third generation. For gDST for Mtb, the Illumina platform may be the most common. The Illumina platform runs genomic DNA extraction, library preparation with DNA fragmentation, ligation of adaptors, adaptor sequencing, and sample enrichment and sequencing using an exclusive device. The actual sequencing step took approximately 2–3 days. This is faster, even considering sample preparation steps, than the conventional pDST.

Currently, an amplicon deep sequencing kit, Deeplex® Myc-TB (Genoscreen, Lille, France), is commercially available [110]. It amplifies 18 target genes and analyzed 13 anti-TB drug resistances: RIF (*rpoB*), INH (*fabG1-inhA*, *katG*, and *ahpC*), PZA (*pncA*), EMB (*embB*), streptomycin (STR: *rrs*, *gidB*, *rpsL*), KM, AMK, CPM (*rrs*), KM (*eis*), CPM (*tlyA*), FQs (*gyrA* and *gyrB*), ETH (*ethA*, *fabG1-inhA*), LZD (*rplC* and *rrl*), BDQ, and CFZ (Rv*0678*). Kambli et al. reported that the gDST results using Deeplex® Myc-TB for 40 sputum specimens yielded 97.5% agreement with pDST [111]. In addition, Cabibbe et al. reported the successful performance of Deeplex® Myc-TB using MinION [112]. The mobile nature of MinION with Deeplex® Myc-TB is a promising technology for the personal use of NGS gDST.

8 Conclusion

Currently, many laboratory examination methods are available for the diagnosis, follow-up, and treatment end point evaluation of TB. It is critical to understand the utility and limitations of each laboratory method, as these methods are useful but involve some complications, which should be addressed in the future.

References

1. Parte AC. LPSN–list of prokaryotic names with standing in nomenclature (bacterio.net), 20 years on. Int J Syst Evol Microbiol. 2018 Jun;68(6):1825–9.
2. Hoefsloot W, Van Ingen J, Andrejak C, Ängeby K, Bauriaud R, Bemer P, et al. The geographic diversity of nontuberculous mycobacteria isolated from pulmonary samples: an NTM-NET collaborative study. Eur Respir J. 2013;42(6):1604–13.
3. Bhat J, Rao V, Muniyandi M, Yadav R, Karforma C, Luke C. Impact of sputum quality and quantity on smear and culture positivity: findings from a tuberculosis prevalence study in Central India. Trans R Soc Trop Med Hyg. 2014;108(1):55–6.
4. Miller DL. A study of techniques for the examination of Sputum in a field survey of chronic bronchitis. Am Rev Respir Dis. 1963 Oct;88:473–83.
5. Alisjahbana B, Van Crevel R, Danusantoso H, Gartinah T, Soemantri ES, Nelwan RHH, et al. Better patient instruction for sputum sampling can improve microscopic tuberculosis diagnosis. Int J Tuberc Lung Dis. 2005;9(7):814–7.
6. Yoon SH, Lee NK, Yim JJ. Impact of sputum gross appearance and volume on smear positivity of pulmonary tuberculosis: a prospective cohort study. BMC Infect Dis [Internet]. 2012;12(1):1. Available from: BMC Infectious Diseases
7. Bastian I, Fujiki A, Gilpin C, Man Kam K, Lumb R. Quality Assurance of Sputum Microscopy in DOTS Programmes countries in the Western Pacific quality Assurance of Sputum Microscopy in DOTS Programmes. Who [Internet]. 2003;45. Available from: http://www.who.int/ihr/training/laboratory_quality/11_cd_rom_tb_eqa_wpro.pdf
8. Thomas TA, Heysell SK, Moodley P, Montreuil R, Ha X, Friedland G, et al. Intensified specimen collection to improve tuberculosis diagnosis in children from rural South Africa, an observational study. BMC Infect Dis. 2014;14(1):11.
9. Sakashita K, Fujita A, Takamori M, Nagai T, Matsumoto T, Saito T, et al. Efficiency of the lung flute for sputum induction in patients with presumed pulmonary tuberculosis. Clin Respir J. 2018;12(4):1503–9.
10. Williams CML, Cheah ESG, Malkin J, Patel H, Otu J, Mlaga K, et al. Face mask sampling for the detection of mycobacterium tuberculosis in expelled aerosols. PLoS One. 2014;9(8):1–7.
11. Williams CM, Abdulwhhab M, Birring SS, De Kock E, Garton NJ, Townsend E, et al. Exhaled mycobacterium tuberculosis output and detection of subclinical disease by face-mask sampling: prospective observational studies. Lancet Infect Dis [Internet]. 2020;20(5):607–17. https://doi.org/10.1016/S1473-3099(19)30707-8.
12. World Health Organization. WHO consolidated guidelines on tuberculosis. Module 3: diagnosis-Rapid diagnostics for tuberculosis detection. https://www.Who.Int/Publications/I/Item/Who-Consolidated-Guidelines-on-Tuberculosis-Module-3-Diagnosis-Rapid-Diagnostics-for-Tuberculosis-Detection. 2020.
13. Sharma S, Shulania A, Achra A, Jeram H, Kansra S, Duggal N. Diagnosis of pulmonary tuberculosis from gastric aspirate samples in nonexpectorating pediatric patients in a tertiary care hospital. Indian J Pathol Microbiol. 2020;63(2):210–3.
14. Singh S, Singh A, Prajapati S, Kabra SK, Lodha R, Mukherjee A, et al. Xpert MTB/RIF assay can be used on archived gastric aspirate and induced sputum samples for sensitive diagnosis of paediatric tuberculosis. BMC Microbiol. 2015;15(1):191.
15. Bates M, O'Grady J, Maeurer M, Tembo J, Chilukutu L, Chabala C, et al. Assessment of the Xpert MTB/RIF assay for diagnosis of tuberculosis with gastric lavage aspirates in children in sub-Saharan Africa: a prospective descriptive study. Lancet Infect Dis. 2013;13(1):36–42.
16. Owens S, Abdel-Rahman IE, Balyejusa S, Musoke P, Cooke RPD, Parry CM, et al. Nasopharyngeal aspiration for diagnosis of pulmonary tuberculosis. Arch Dis Child. 2007;92(8):693–6.
17. Zar HJ, Workman LJ, Prins M, Bateman LJ, Mbhele SP, Whitman CB, et al. Tuberculosis diagnosis in children using Xpert ultra on different respiratory specimens. Am J Respir Crit Care Med. 2019;200(12):1531–8.

18. Oramasionwu GE, Heilig CM, Udomsantisuk N, Kimerling ME, Eng B, Nguyen HD, et al. The utility of stool cultures for diagnosing tuberculosis in people living with the human immunodeficiency virus. Int J Tuberc Lung Dis. 2013;17(8):1023–8.
19. Walters E, Demers AM, Van Der Zalm MM, Whitelaw A, Palmer M, Bosch C, et al. Stool culture for diagnosis of pulmonary tuberculosis in children. J Clin Microbiol. 2017;55(12):3355–65.
20. MacLean E, Sulis G, Denkinger CM, Johnston JC, Pai M, Khana FA. Diagnostic accuracy of stool Xpert MTB/RIF for detection of pulmonary tuberculosis in children: a systematic review and meta-analysis. J Clin Microbiol. 2019;57(6):1–12.
21. Kabir S, Rahman SMM, Ahmed S, Islam MS, Banu RS, Shewade HD, et al. Xpert ultra assay on stool to diagnose pulmonary tuberculosis in children. Clin Infect Dis. 2020;73(2):1–9.
22. Ghosh HK, Cobb M, Pacey DP, Conklin S. Experience with a simplification of the Petroff method for laboratory diagnosis of mycobacteria in Sputum. Pathology [Internet]. 1978;10(3):257–61. Available from: https://www.tandfonline.com/doi/abs/10.3109/00313027809063509
23. de Kantor INLA. In: GangadharamP PRJ, Jenkins A, editors. Tuberculosis: laboratory procedure for developing countries. New Tork: Chapman Hall ITP; 1998. p. 351–9.
24. Aldini G, Altomare A, Baron G, Vistoli G, Carini M, Borsani L, et al. N-Acetylcysteine as an antioxidant and disulphide breaking agent: the reasons why. Free Radic Res [Internet]. 2018;52(7):751–62. https://doi.org/10.1080/10715762.2018.1468564.
25. Plongla R, Preece CL, Perry JD, Gilligan PH. Evaluation of RGM medium for isolation of nontuberculous mycobacteria from respiratory samples from patients with cystic fibrosis in the United States. J Clin Microbiol. 2017;55(5):1469–77.
26. Asmar S, Drancourt M. Chlorhexidine decontamination of sputum for culturing mycobacterium tuberculosis. BMC Microbiol [Internet]. 2015;15(1):4–9. https://doi.org/10.1186/s12866-015-0479-4.
27. Kent PT, Kubica GP. Public health mycobacteriology: a guide for the level III Laboratory. 1985;
28. Ratnam S, March SB. Effect of relative centrifugal force and centrifugation time on sedimentation of mycobacteria in clinical specimens. J Clin Microbiol. 1986;23(3):582–5.
29. Yoshimatsu S, Kato-Matsumaru T, Aono A, Chikamatsu K, Yamada H, Mitarai S. Factors contribute to efficiency of specimen concentration of mycobacterium tuberculosis by centrifugation and magnetic beads. Int J Mycobacteriol. 2015;4(3):245–9.
30. Asmar S, Chatellier S, Mirande C, Van Belkum A, Canard I, Raoult D, et al. A novel solid medium for culturing mycobacterium tuberculosis isolates from clinical specimens. J Clin Microbiol. 2015;53(8):2566–9.
31. Ghodbane R, Raoult D, Drancourt M. Dramatic reduction of culture time of mycobacterium tuberculosis. Sci Rep. 2014;4:4–7.
32. Mullis K, Faloona F, Scharf S, Saiki R, Horn G, Erlich H. Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction. Cold Spring Harb Symp Quant Biol. 1986;51(Pt 1):263–73.
33. Moore DF, Curry JI, Knott CA, Jonas V. Amplification of rRNA for assessment of treatment response of pulmonary tuberculosis patients during antimicrobial therapy. J Clin Microbiol. 1996;34(7):1745–9.
34. Tsara V, Serasli E, Christaki P. Problems in diagnosis and treatment of tuberculosis infection. Hippokratia. 2009;13(1):20–2.
35. Helb D, Jones M, Story E, Boehme C, Wallace E, Ho K, et al. Rapid detection of mycobacterium tuberculosis and rifampin resistance by use of on-demand, near-patient technology. J Clin Microbiol. 2010;48(1):229–37.
36. Lawn SD, Nicol MP. Erratum: Xpert® MTB/RIF assay: development, evaluation and implementation of a new rapid molecular diagnostic for tuberculosis and rifampicin resistance. (Future Microbiology (2011) 6:9 (1067-1082)). Future Microbiol. 2012;7(8):1024.

37. Steingart KR, Sohn H, Schiller I, Kloda LA, Boehme CC, Pai M, Dendukuri N. Xpert® MTB/RIF assay for pulmonary tuberculosis and rifampicin resistance in adults. Cochrane Database Syst Rev. 2013;31(1):CD009593. https://doi.org/10.1002/14651858.CD009593.pub2. Update in: Cochrane Database Syst Rev. 2014;(1):CD009593. PMID: 23440842; PMCID: PMC4470352.
38. Horne D, Kohli M, Zifodya J, Schiller I, Dendukuri N, Tollefson D, et al. Horne DJ, Kohli M, Zifodya JS, Schiller I, Dendukuri N, Tollefson D, Schumacher SG, Ochodo EA, Pai M, Steingart KR. Cochrane Database Syst Rev 2019;(6).
39. Sohn H, Aero AD, Menzies D, Behr M, Schwartzman K, Alvarez GG, et al. Xpert MTB/RIF testing in a low tuberculosis incidence, high-resource setting: limitations in accuracy and clinical impact. Clin Infect Dis. 2014;58(7):970–6.
40. Tsuyuguchi K, Nagai H, Ogawa K, Matsumoto T, Morimoto K, Takaki A, et al. Performance evaluation of Xpert MTB/RIF in a moderate tuberculosis incidence compared with TaqMan MTB and TRCRapid M.TB. J Infect Chemother. 2017;23(2):101–6.
41. Zong K, Luo C, Zhou H, Jiang Y, Li S. Xpert MTB/RIF assay for the diagnosis of rifampicin resistance in different regions: a meta-analysis. BMC Microbiol. 2019;19(1):1–21.
42. WHO. Automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampicin resistance : xpert MTB/Rif System policy statement. world health [Internet]. 2011;1–35. Available from: http://www.stoptb.org/wg/gli/assets/documents/XpertImplementationDocument.pdf
43. World Health Organisation. Guidelines for the programmatic management of. 2011.
44. WHO. Companion handbook. World Health Organization. 2014. 464.
45. Jaleta KN, Gizachew M, Gelaw B, Tesfa H, Getaneh A, Biadgo B. Rifampicin-resistant Mycobacterium tuberculosis among tuberculosis-presumptive cases at University of Gondar hospital, Northwest Ethiopia. Infect Drug Resist. 2017;10:185–92.
46. Traore H, Fissette K, Bastian I, Devleeschouwer M, Portaels F. Detection of rifampicin resistance in mycobacterium tuberculosis isolates from diverse countries by a commercial line probe assay as an initial indicator of multidrug resistance. Int J Tuberc Lung Dis. 2000;4(5):481–4.
47. Kenaope L, Ferreira H, Seedat F, Otwombe K, Martinson NA, Variava E. Sputum culture and drug sensitivity testing outcome among X-pert Mycobacterium tuberculosis/rifampicin-positive, rifampicin-resistant sputum: a retrospective study–not all rifampicin resistance is multi-drug resistant. J Glob Antimicrob Resist [Internet]. 2020;21:434–8. https://doi.org/10.1016/j.jgar.2019.11.008.
48. Mathys V, Van De Vyvere M, De Droogh E, Soetaert K, Groenen G. False-positive rifampicin resistance on Xpert®MTB/RIF caused by a silent mutation in the rpoB gene. Int J Tuberc Lung Dis. 2014;18(10):1255–7.
49. Dorman SE, Schumacher SG, Alland D, Nabeta P, Armstrong DT, King B, et al. Xpert MTB/RIF ultra for detection of mycobacterium tuberculosis and rifampicin resistance: a prospective multicentre diagnostic accuracy study. Lancet Infect Dis. 2018;18(1):76–84.
50. Plannning for country to Xpert MTB/RIF Ultra.
51. World Health Organization. Meeting report of a technical expert consultation : non-inferiority analysis of Xpert MTB/RIF Ultra compared to Xpert MTB/RIF 2017;1–11. Available from: http://apps.who.int/bookorders.
52. Feuerriegel S, Kohl TA, Utpatel C, Andres S, Maurer FP, Heyckendorf J, et al. Rapid genomic first- and second-line drug resistance prediction from clinical *mycobacterium tuberculosis* specimens using Deeplex®-MycTB. Eur Respir J [Internet]. 2020;1:2001796. Available from: http://erj.ersjournals.com/content/early/2020/06/25/13993003.01796-2020.abstract
53. Sputum M, Cao Y, Parmar H, Gaur RL, Lieu D, Raghunath S, et al. Xpert MTB/XDR : a 10-Color Re fl Ex assay suitable for point-of- care settings to detect isoniazid, fluoroquinolone, and second-line-injectable-drug resistance directly from.
54. Notomi T, Okayama H, Masubuchi H, Yonekawa T, Watanabe K, Amino N, et al. Loop-mediated isothermal amplification of DNA. Nucleic Acids Res. 2000;28(12):E63.

55. Mitarai S, Okumura M, Toyota E, Yoshiyama T, Aono A, Sejimo A, et al. Evaluation of a simple loop-mediated isothermal amplification test kit for the diagnosis of tuberculosis. Int J Tuberc Lung Dis. 2011;15(9):1211–7.
56. World Health Organization. The use of loop-mediated isothermal amplification (TB-LAMP) for the diagnosis of pulmonary tuberculosis: policy guidance. 2016.
57. Shete PB, Farr K, Strnad L, Gray CM, Cattamanchi A. Diagnostic accuracy of TB-LAMP for pulmonary tuberculosis: a systematic review and meta-analysis. BMC Infect Dis. 2019;19(1):1–29.
58. Nakiyingi L, Nakanwagi P, Briggs J, Agaba T, Mubiru F, Mugenyi M, et al. Performance of loop-mediated isothermal amplification assay in the diagnosis of pulmonary tuberculosis in a high prevalence TB/HIV rural setting in Uganda. BMC Infect Dis. 2018;18(1):1–10.
59. Nliwasa M, MacPherson P, Chisala P, Kamdolozi M, Khundi M, Kaswaswa K, et al. The sensitivity and specificity of loop-mediated isothermal amplification (LAMP) assay for tuberculosis diagnosis in adults with chronic cough in Malawi. PLoS One [Internet]. 2016;11(5):1–13. https://doi.org/10.1371/journal.pone.0155101.
60. WHO. Rapid Communication : molecular assays as initial tests for the diagnosis of tuberculosis and rifampicin resistance. World Heal Organ [Internet]. 2020; Policy upd (January):1–8. Available from: http://apps.who.int/bookorders.
61. Nikam C, Kazi M, Nair C, Jaggannath M, Manoj MM, Vinaya RV, et al. Evaluation of the Indian TrueNAT micro RT-PCR device with GeneXpert for case detection of pulmonary tuberculosis. Int J Mycobacteriol [Internet]. 2014;3(3):205–10. https://doi.org/10.1016/j.ijmyco.2014.04.003.
62. Seifert M, Ajbani K, Georghiou SB, Catanzaro D, Rodrigues C, Crudu V, et al. A performance evaluation of MTBDRplus version 2 for the diagnosis of multidrug-resistant tuberculosis. Int J Tuberc Lung Dis. 2016;20(5):631–7.
63. World Health Organization. The use of molecular line probe assays for the detection of resistance to isoniazid and rifampicin Policy update. 2016; Available from: https://www.libnauka.ru/item.php?doi=10.7868/S2587667820060357
64. WHO. The use of molecular line probe assays for the detection of resistance to second-line anti-tuberculosis drugs. Policy guidance. WHO/HTM/TB/2016.07. Geneva, Switzerland: World Health Organization; 2016.
65. World Health Organization. Meeting report of the WHO expert consultation on the definition of extensively drug-resistant tuberculosis [Internet]. 2020. CC BY-NC-SA 3.0 IGO. Available from: https://www.who.int/publications/i/item/meeting-report-of-the-who-expert-consultation-on-the-definition-of-extensively-drug-resistant-tuberculosis
66. Bai Y, Wang Y, Shao C, Hao Y, Jin Y. GenoType MTBDRplus assay for rapid detection of multidrug resistance in mycobacterium tuberculosis: a meta-analysis. PLoS One [Internet]. 2016;11(3):1–20. https://doi.org/10.1371/journal.pone.0150321.
67. Tomasicchio M, Theron G, Pietersen E, Streicher E, Stanley-Josephs D, Van Helden P, et al. The diagnostic accuracy of the MTBDRplus and MTBDRsl assays for drug-resistant TB detection when performed on sputum and culture isolates. Sci Rep. 2016;6(November 2015):1–8.
68. Gardee Y, Dreyer AW, Koornhof HJ, Omar SV, Silva P, Bhyat Z. Evaluation of the GenoType MTBDRsl tuberculosis isolates in South Africa. J Clin Microbiol [Internet]. 2017;55(3):791–800. https://doi.org/10.1128/JCM.01865-16.
69. Willby MJ, Wijkander M, Havumaki J, Johnson K, Werngren J, Hoffner S, et al. Detection of mycobacterium tuberculosis pncA mutations by the Nipro Genoscholar PZA-TB II assay compared to conventional sequencing. Antimicrob Agents Chemother. 2018 Jan;62(1):e01871–17.
70. Aono A, Chikamatsu K, Yamada H, Kato T, Mitarai S. Association between pncA gene mutations, pyrazinamidase activity, and pyrazinamide susceptibility testing in mycobacterium tuberculosis. Antimicrob Agents Chemother. 2014;58(8):4928–30.

71. Aono A, Chikamatsu K, Yamada H, Igarashi Y, Murase Y, Takaki A, et al. A simplified pyrazinamidase test for pyrazinamide drug susceptibility in mycobacterium tuberculosis. J Microbiol Methods [Internet]. 2018;154(October):52–4. https://doi.org/10.1016/j.mimet.2018.09.018.
72. O'Connor JA, O'Reilly B, Corcoran GD, O'Mahony J, Lucey B. A comparison of the HAIN Genotype CM reverse hybridisation assay with the Bruker MicroFlex LT MALDI-TOF mass spectrometer for identification of clinically relevant mycobacterial species. Br J Biomed Sci. 2020 Jul;77(3):152–5.
73. Springer B, Stockman L, Teschner K, Roberts GD, Bottger EC. Two-laboratory collaborative study on identification of mycobacteria: molecular versus phenotypic methods. J Clin Microbiol. 1996;34(2):296–303.
74. Kazumi Y, Mitarai S. The evaluation of an identification algorithm for mycobacterium species using the 16S rRNA coding gene and rpoB. Int J Mycobacteriol. 2012;1(1):21–8.
75. Alcolea-Medina A, Fernandez MTC, Montiel N, García MPL, Sevilla CD, North N, et al. An improved simple method for the identification of mycobacteria by MALDI-TOF MS (matrix-assisted laser desorption- ionization mass spectrometry). Sci Rep. 2019;9(1):5–10.
76. Tasaka H, Shigeto E, Matsuo K, Yamaguchi R, Haga S, Yamazaki A, et al. Secretion of MPB64 antigen by a recombinant clone of mycobacterium smegmatis: characterization and application for the diagnosis of tuberculosis. Scand J Immunol. 1995 Oct;42(4):487–92.
77. Chikamatsu K, Aono A, Yamada H, Sugamoto T, Kato T, Kazumi Y, et al. Comparative evaluation of three immunochromatographic identification tests for culture confirmation of mycobacterium tuberculosis complex. BMC Infect Dis. 2014;14(1):54.
78. Hirano K, Aono A, Takahashi M, Abe C. Mutations including IS6110 insertion in the gene encoding the MPB64 protein of Capilia TB-negative mycobacterium tuberculosis isolates. J Clin Microbiol. 2004;42(1):390–2.
79. Orikiriza P, Nyehangane D, Atwine D, Kisakye JJ, Kassaza K, Amumpaire J-M, et al. Evaluation of the SD Bioline TB ag MPT64 test for identification of *mycobacterium tuberculosis* complex from liquid cultures in southwestern Uganda. Afr J Lab Med. 2017;6(2):1–4.
80. Said HM, Ismail N, Osman A, Velsman C, Hoosen AA. Evaluation of TBc identification immunochromatographic assay for rapid identification of mycobacterium tuberculosis complex in samples from broth cultures. J Clin Microbiol. 2011;49(5):1939–42.
81. Saifi M, Jabbarzadeh E, Bahrmand AR, Karimi A, Pourazar S, Fateh A, et al. HSP65-PRA identification of non-tuberculosis mycobacteria from 4892 samples suspicious for mycobacterial infections. Clin Microbiol Infect. 2013;19(8):723–8.
82. Macheras E, Roux AL, Bastian S, Leão SC, Palaci M, Sivadon-Tardy V, et al. Multilocus sequence analysis and rpoB sequencing of mycobacterium abscessus (sensu lato) strains. J Clin Microbiol. 2011;49(2):491–9.
83. Matsumoto Y, Kinjo T, Motooka D, Nabeya D, Jung N, Uechi K, et al. Comprehensive subspecies identification of 175 nontuberculous mycobacteria species based on 7547 genomic profiles. Emerg Microbes Infect. 2019;8(1):1043–53.
84. Brock I, Weldingh K, Leyten EMS, Arend SM, Ravn P, Andersen P. Specific T-cell epitopes for immunoassay-based diagnosis of mycobacterium tuberculosis infection. J Clin Microbiol. 2004;42(6):2379–87.
85. Liu C, Zhao Z, Fan J, Lyon CJ, Wu H-J, Nedelkov D, et al. Quantification of circulating Mycobacterium tuberculosis and antigen peptides allows rapid diagnosis of active disease and treatment monitoring. Proc Natl Acad Sci [Internet]. 2017;114(15):3969 LP–3974. Available from: http://www.pnas.org/content/114/15/3969.abstract
86. Liu C, Lyon CJ, Bu Y, Deng Z, Walters E, Li Y, et al. Clinical evaluation of a blood assay to diagnose paucibacillary tuberculosis via bacterial antigens. Clin Chem. 2018;64(5):791–800.
87. World Health Organization. The use of lateral flow urine lipoarabinomannan assay (LF-LAM) for the diagnosis and screening of active tuberculosis in people living with HIV Policy guidance. 2015;(October).

88. Broger T, Nicol MP, Sigal GB, Gotuzzo E, Zimmer AJ, Surtie S, et al. Diagnostic accuracy of 3 urine lipoarabinomannan tuberculosis assays in HIV-negative outpatients. J Clin Invest. 2020;130(11):5756–64.
89. Broger T, Tsionksy M, Mathew A, Lowary TL, Pinter A, Plisova T, et al. Sensitive electrochemiluminescence (ECL) immunoassays for detecting lipoarabinomannan (LAM) and ESAT-6 in urine and serum from tuberculosis patients. PLoS One. 2019;14(4):1–19.
90. Kawasaki M, Echiverri C, Raymond L, Cadena E, Reside E, Gler MT, et al. Lipoarabinomannan in sputum to detect bacterial load and treatment response in patients with pulmonary tuberculosis: analytic validation and evaluation in two cohorts. PLoS Med. 2019;16(4):e1002780.
91. Sakashita K, Takeuchi R, Takeda K, Takamori M, Ito K, Igarashi Y, et al. Ultrasensitive enzyme-linked immunosorbent assay for the detection of MPT64 secretory antigen to evaluate mycobacterium tuberculosis viability in sputum. Int J Infect Dis [Internet]. 2020;96:244–53. https://doi.org/10.1016/j.ijid.2020.04.059.
92. Kim SJ. Drug-susceptibility testing in tuberculosis: methods and reliability of results. Eur Respir J. 2005;25(3):564–9.
93. Kam KM, Sloutsky A, Yip CW, Bulled N, Seung KJ, Zignol M, et al. Determination of critical concentrations of second-line anti-tuberculosis drugs with clinical and microbiological relevance. Int J Tuberc Lung Dis. 2010;14(3):282–8.
94. Schön T, Miotto P, Köser CU, Viveiros M, Böttger E, Cambau E. Mycobacterium tuberculosis drug-resistance testing: challenges, recent developments and perspectives. Clin Microbiol Infect. 2017;23(3):154–60.
95. World Health Organization. Technical Report on critical concentrations for drug susceptibility testing of medicines used in the treatment of drug-resistant tuberculosis. 2018.
96. Gail L. Woods. Performance standards for susceptibility testing of Mycobacteria, Nocardia spp., and other aerobic actinomycetes, 1st Edition. 2018. 36 p.
97. Goloubeva V, Lecocq M, Lassowsky P, Matthys F, Portaels F, Bastian I. Evaluation of mycobacteria growth indicator tube for direct and indirect drug susceptibility testing of mycobacterium tuberculosis from respiratory specimens in a Siberian prison hospital. J Clin Microbiol. 2001;39(4):1501–5.
98. Somoskovi A, Clobridge A, Larsen SC, Sinyavskiy O, Surucuoglu S, Parsons LM, et al. Does the MGIT 960 system improve the turnaround times for growth detection and susceptibility testing of the mycobacterium tuberculosis complex? J Clin Microbiol. 2006;44(6):2314–5.
99. Moore DAJ, Evans CAW, Gilman RH, Caviedes L, Coronel J, Vivar A, et al. Microscopic-observation drug-susceptibility assay for the diagnosis of TB. N Engl J Med. 2006;355(15):1539–50.
100. Wikman-Jorgensen P, Llenas-García J, Hobbins M, Ehmer J, Abellana R, Goncalves AQ, et al. Microscopic observation drug susceptibility assay for the diagnosis of TB and MDR-TB in HIV-infected patients: a systematic review and meta-analysis. Eur Respir J. 2014;44(4):973–84.
101. Moore D. MODS–a user guide Microscopic observation drug susceptibility assay. 2008;MODS user(November):30.
102. Mejia GI, Castrillon L, Trujillo H, Robledo JA. Microcolony detection in 7H11 thin layer culture is an alternative for rapid diagnosis of mycobacterium tuberculosis infection. Int J Tuberc Lung Dis. 1999;3(2):138–42.
103. Ardizzoni E, Ariza E, Mulengwa D, Mpala Q, de La Tour R, Maphalala G, et al. Thin-layer-agar-based direct phenotypic drug susceptibility testing on Sputum in Eswatini rapidly detects mycobacterium tuberculosis growth and rifampicin resistance otherwise missed by WHO-endorsed diagnostic tests. Antimicrob Agents Chemother. 2021;65(6):e02263–20.
104. World Health Organization. The use of next-generation sequencing technologies for the detection of mutations associated with drug resistance in mycobacterium tuberculosis complex: technical guide. Geneva: WHO/CDS/TB; 2018.

105. Coll F, McNerney R, Preston MD, Guerra-Assunção JA, Warry A, Hill-Cawthorne G, et al. Rapid determination of anti-tuberculosis drug resistance from whole-genome sequences. Genome Med [Internet]. 2015;7(1):1–10.
106. Campbell EA, Korzheva N, Mustaev A, Murakami K, Nair S, Goldfarb A, et al. Structural mechanism for rifampicin inhibition of bacterial RNA polymerase. Cell. 2001;104(6):901–12.
107. Mani C, Selvakumar N, Narayanan S, Narayanan PR. Mutations in the rpoB gene of multidrug-resistant mycobacterium tuberculosis clinical isolates from India. J Clin Microbiol. 2001;39(8):2987–90.
108. Farhat MR, Sixsmith J, Calderon R, Hicks ND, Fortune SM, Murray M. Rifampicin and rifabutin resistance in 1003 mycobacterium tuberculosis clinical isolates. J Antimicrob Chemother. 2019;74(6):1477–83.
109. Van Deun A, Aung KJM, Bola V, Lebeke R, Hossain MA, De Rijk WB, et al. Rifampin drug resistance tests for tuberculosis: challenging the gold standard. J Clin Microbiol. 2013;51(8):2633–40.
110. Jouet A, Gaudin C, Badalato N, Allix-Béguec C, Duthoy S, Ferré A, et al. Deep amplicon sequencing for culture-free prediction of susceptibility or resistance to 13 anti-tuberculous drugs. Eur Respir J. 2021;57(3):2002338.
111. Kambli P, Ajbani K, Kazi M, Sadani M, Naik S, Shetty A, et al. Targeted next generation sequencing directly from sputum for comprehensive genetic information on drug resistant mycobacterium tuberculosis. Tuberculosis. 2021;127(January):102051.
112. Cabibbe AM, Spitaleri A, Battaglia S, Colman RE, Suresh A, Uplekar S, et al. Application of targeted next-generation sequencing assay on a portable sequencing platform for culture-free detection of drug-resistant tuberculosis from clinical samples. J Clin Microbiol. 2020;58(10):1–9.

Part II
Pulmonary TB

Chapter 5
Advances in Diagnostics of Pulmonary TB: What Is the Latest Approach to Diagnose Pulmonary TB?

Yuka Sasaki

Abstract The gold standard for diagnosing pulmonary TB is to detect *Mycobacterium tuberculosis* (M. TB) in a human specimen. Case detection methods vary depending on the medical resources and economic conditions in each region. These methods can be either passive, in which case the disease is diagnosed after the patient has had a consultation, or active, when infections are detected via screening and medical examination. In countries with low TB prevalence, passive case detection is the primary method used, and clinicians deal with many other diseases as well, so various tests have been developed to diagnose pulmonary TB and other diseases. Passive case detection is used in areas where TB is widespread and in populations in which infections are particularly common, especially when medical resources, efficient diagnostic methods, and commonly used tests are limited in the area. Thus, the choice of diagnostic method used depends strongly on the local situation. The number of TB patients worldwide is gradually declining in most countries. To accelerate the decline in infections, the World Health Organization (WHO) has devised various measures for bacterial testing in the focal region. In 2021, the WHO once again recommended rapid and accurate sputum testing to increase the number of patients for whom treatment is initiated and to accelerate and improve the efficiency of TB diagnosis. This is being done with the aim of reducing the number of patients, deaths, and cases with multidrug-resistant TB not just drug-sensitive TB. The WHO has recommended converting from the conventional smear method to the nucleic acid amplification test (NAAT). As culture tests are expensive, it is recommended that patients be triaged first via symptomatic screening and digital imaging tests to reduce the number of target patients, after which the NAAT should be performed. For this triage process, testing methods using human specimens that can be obtained more easily are under development. There are many benefits to early detection of pulmonary TB and rifampicin (RFP)-resistant cases. However, in countries where the prevalence of TB has declined, the diagnosis of

Y. Sasaki (✉)
Center for Pulmonary Disease, National Hospital Organization Tokyo National Hospital, Kiyose, Japan

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other diseases is also important, so approaches such as bronchoscopy and positron emission tomography (PET) are used for patients who cannot be diagnosed using the NAAT. The onset of TB in people living with HIV (PLWHIV) has become a major problem of this coinfection, regardless of the local prevalence of TB. Lipoarabinomannan has been developed as a biomarker for this population, and since it uses an easily available sample (urine), it is used both in the triage process and in the low-prevalence areas. Many diagnostic methods will be developed in the future, but the latest approach to diagnosing TB is to suspect it and then perform a NAAT.

Keywords Pulmonary TB · Diagnosis · NAAT · Developing countries Developed countries

1 Introduction

TB is a chronic bacterial infectious disease and is one of the three major and harmful infectious diseases in the world.

Globally, an estimated 10.0 million (range, 8.9–11.0 million) people fell ill with TB in 2019, and there were an estimated 1.2 million (range, 1.1–1.3 million) TB deaths among HIV-negative people in 2019, and an additional 208,000 deaths (range, 177, 000–242, 000) among people living with Human immunodeficiency virus (HIV). Among all those affected with TB, 8.2% were people living with HIV (PLWHIV) [1].

TB is usually cured with standard anti-TB treatment. Drug resistance, coinfection with HIV, comorbidities, and deterioration due to long delay in case detection exacerbate the prognosis. The gold standard for the diagnosis of TB is the detection of *Mycobacterium tuberculosis* (M. TB), but the culture test may take some weeks more. In resource-limited countries, some TB patients need a long time to be initiated anti-TB treatment. An accurate diagnosis is not always made promptly in many places of the world.

WHO has set a goal of 40 million people to be treated for TB between 2018 and 2022, but in 2018 and 2019, only 14 million people, 35% of the target, were treated.

Many TB strategies have been planned, but the number of patients has not been reduced sufficiently. One of the reasons was the problem of insufficient diagnosis of patients. Finding TB patients may not be easy even in countries with ample medical resources. In resource-poor countries, some serious problems have been pointed out. The first is that patients cannot access a medical institution due to the scarcity of medical institutions and the underdeveloped transportation system, and the second is that patients do not seek medical facilities because they have no or mild symptoms. The third is that the diagnosis of TB is poorly accurate and can lead to misdiagnosis. Finally, the number of TB cases is not reported correctly in some countries, which is related to problems with the administrative reporting system. WHO reported despite increases in TB notifications, there was still a large gap (2.9

million) between the number of people newly diagnosed and reported and the ten million people estimated to have developed TB in 2019, and this gap is due to a combination of underreporting of people diagnosed with TB and underdiagnosis.

There are special difficulties in diagnosing TB in PLWHIV and drug-resistant TB. HIV infection causes decrease in cell-mediated immunity, occurring TB more likely. It has been reported that the number of HIV, TB coinfected cases are numerous, especially in the sub-Saharan African region. When diagnosing TB, the patient should be tested for HIV infection. In addition, HIV patients should continue to be tested for TB infection.

Drug-resistant TB has become a very big problem. Drug-resistant TB, especially multi-drug resistant tuberculosis (MDR-TB), which is resistant to both isoniazid and rifampicin, cannot be cured without long-term oral administration of many mild effective drugs. In 2019, widespread drug-resistant TB was reported in more than 100 countries. WHO estimates [1] that close to half a million people developed rifampicin-resistant TB (RR-TB), of which 78% had MDR-TB. The three countries with the largest share of the global burden were India (27%), China (14%), and the Russian Federation (8%) in 2019. MDR-TB has been produced by the administration of inappropriate anti-TB drugs, so in the future, MDR-TB may delay the eradication of TB, if not tightly controlled and properly treated. Recently, short-term treatments with a small number of new treatments are being considered [2], but the days of such treatments around the world are yet to come. Early diagnosis of MDR-TB is very important because MDR-TB also spreads due to airborne infections.

Diagnosis of TB in children is also important in countries with high TB burden. They may not be able to be examined as same as adults. If child mortality will be reduced, the population that develops the region would increase. In addition, if the disability caused by tuberculous sequelae will be reduced, the mother would be able to grow her children with peace of mind.

This chapter describes the approaches for diagnosing pulmonary tuberculosis (pulmonary TB), but it should be noted that the approach to diagnosis differs between areas with high and low TB burden. It should also be noted that there may be differences in the optimum methods that can be used in economically favorable countries with abundant medical resources and developing countries. However, it is important and common in all the countries regardless of availability of medical resources that diagnosis of pulmonary TB is based on the detection of M.TB and the confirmation of drug resistance, especially that of rifampicin.

2 Symptoms

TB is divided into primary TB and secondary (post-primary) TB depending on the period from infection to onset of disease. In primary TB, following the initial infection, tubercle bacilli continue to proliferate developing lesions in the lung, the lymph node and the pleural space, and sometimes spread into the bloodstream to

cause miliary TB (disseminated TB). When pulmonary TB develops in primary TB, the hilum lymph nodes often get swelling. In the case of miliary TB involving various organs such as the brain and the pleura fever and other characteristic signs and symptoms caused by each involved organ may be seen. Secondary TB is caused by the bacilli which have been suppressed by host's immunity (endogenous reactivation). Pulmonary TB is the most common form of secondary TB.

It is not uncommon for patients to be diagnosed from the symptoms and medical examinations. Symptoms of pulmonary TB include fever, weight loss, and night sweats as systemic symptoms, and cough and sputum as respiratory symptoms. However, since these symptoms are not specific to pulmonary TB, it is difficult to diagnose pulmonary TB from these symptoms. It is not uncommon for all of these symptoms to appear. Often, these symptoms are mild for many months, thus leading to delays in a patient's seeking care and increasing the risk of spreading the infection to others.

Other systemic symptoms may include emaciation, malaise, and loss of appetite, and as TB progresses, additional complaints can occur, such as bloody sputum, shortness of breath, chest pain and dyspnea, and massive hemoptysis.

Cough is the predominant symptom of pulmonary TB, and patients with chronic cough lasting for longer than 2 weeks should be suspected of having Pulmonary TB. In a recent article [3], 72.7% of Pulmonary TB patients had cough and in 48.2% of patients it had been lasting for >2 weeks. In WHO review [4], cough lasting for 2–3 weeks also showed pooled sensitivity of 35% (95% confidence interval: 24–46%), and pooled specificity (95% CI: 93–97%). Cough, regardless of duration, showed 57% pooled sensitivity (95% CI: 40–74%) and 80% pooled specificity (95% CI: 69–90%). A recent systematic review [5] also admits that even in high prevalence countries, patients who have cough lasting for >2 weeks do not always have pulmonary TB. In this review, thus, it is recommended to suspect Pulmonary TB when people with cough are at high risk of TB, e.g., residents of areas with a high prevalence of TB, prisoners, people living with HIV [PLWHIV], and contacts of infectious pulmonary TB.

In children or elders, they might only complain fever, loss of appetite, malaise, and weight loss without cough. Nonspecific complaints of these people cause long delays in diagnosis. Findings of physical examination of Pulmonary TB are not specific and are not characteristic of mild pulmonary TB. In severe cases, cyanosis due to hypoxemia an emaciation may be observed. On auscultation, post-tussive crackles may be heard during inspiration. If the lung tissue is widely damaged, the respiratory sounds may be weakened. Wheezing can be heard when bronchial TB lesion is present or when the bronchi are narrowed. When pleural effusion is present, dull sounds on percussion and diminished breath sounds are observed on the side with pleural effusion. Palpation of superficial lymph nodes is important. In patients with unknown HIV infection, superficial lymphadenopathy may trigger the diagnosis of both TB and HIV infections.

3 Delays

Early diagnosis and prompt treatment are the minimum requirements to control TB burden. Limited knowledge about TB and misdiagnosis in health facilities result in delays in pulmonary TB diagnosis and treatment. As a result, delays increase the risk of TB transmission within the community, severity, and mortality of patients. Pulmonary TB presents nonspecific symptoms and might be easily confused with other common respiratory infectious diseases.

WHO definite delays are as follows: patient delay is duration of the interval between onset of symptom and patient's presentation to a health care provider; health care system delay is duration of interval between the date of health-seeking behavior at a health care provider and the initiation of anti-TB treatment, and total delay is the sum of patient and health care system delay [6].

According to a meta-analysis of studies in Asia [7], the main factors of the duration of patient delay were male sex, unemployment, low-income level, hemoptysis, positive sputum smears, chest radiographs, and long travel time or distance to the first health care providers. Male sex and hemoptysis showed negative correlation, and the others positive correlation. Positive sputum smear and imaging tests may be results of long patient delays, and the indifference to the presence of long-lasting cough or sputum.

According to an examination in Italy [8], factors contributing to patient delay can be: sociodemographic, physical, financial, health literacy, religious-cultural and stigma. The cause of patient delay depends on medical resources and economic background, hence patient delay should be addressed according to region.

Regarding the provider delay, it may occur in the health system. Health system delay (HSD)-related factors are poor TB knowledge among health care providers, lack of effective diagnostic tools, clinicians with little experience in TB diagnosis, and system error in the medical services. Quattrocchi et al. [8] reported factors associated with long doctor delay (>7 days) were the same as those associated with HSD, and in addition, having cough for >3 weeks was significantly associated with shorter doctor delay [8].

In Japan's TB surveillance, a delay survey for pulmonary TB diagnosis has been conducted since 1987, and it has been reported as patient's delay, doctor's delay, and total delay, which is the sum of the former two delays. The indicator of patient's delay is "% of patients who have visited a doctor for the first time later than 2 months after the onset of pulmonary TB." The indicator of doctor's delay is "% of patients whom it took more than 1 month until the diagnosis of TB from the first visit to the doctor(s)." Many studies about delay have been conducted in Japan with the aim of reducing the number of TB patients detected late after the onset [9]. These studies showed that patient's delay was caused mainly due to poor consciousness of health, and masked symptoms by smoking. Doctor's delay was caused by the delayed chest X-ray examination and/or sputum examination at medical institutions due to doctors' low index of suspect of TB and selecting the wrong algorithm in diagnosis.

In economically developed countries, pulmonary TB patients have been decreasing, the encountering TB patients at medical institutions have also been decreasing, and even with advanced medical equipment, the health system delay may not be shortened when health facilities workers have poor knowledge to diagnose pulmonary TB.

In high TB burden countries, the duration of health system delay may be shorter. The clinicians have enough knowledge and awareness about pulmonary TB, because pulmonary TB is one of the most important enemies for them and quick TB diagnosis is always required. However, health system delays may be longer when medical resources are scarce and test results cannot be obtained promptly. In addition, if the patient fails to return to the clinic for the test result, due to problems such as transportation, then the start of treatment will be delayed, leading to another delay, i.e., start of treatment delay.

Early consultation and early diagnosis are important factors in improving TB control. In order to reduce these delays, it is necessary to take measures according to the local conditions.

4 TB Diagnostic Methods

4.1 Conventional Examinations

Upon initial pulmonary TB diagnosis, sputum examination is the most critical and microscopic sputum smear examination is the most widely used method globally. When a clinician suspects that a patient has pulmonary TB based on medical history and symptoms, a sputum test and chest imaging are often carried out, with both examinations often being carried out together. A sputum test can be used not only to diagnose pulmonary TB but also to assess the patient's infectiousness and susceptibility to anti-TB drugs. If it is possible to conduct a culture test at the same time, the MTB strain can be isolated and tested for susceptibility and subjected to genomic analysis.

However, not all patients are able to expectorate sputum readily. Poor quality specimen leads to misdiagnosis, and quality assurance of specimens is extremely important. After the sputum quality has been confirmed, a smear examination is performed via carbon fuchsin staining or fluorescent staining (Ziehl-Neelsen staining or Kinyoun staining). If sputum cannot be obtained, airway samples such as gastric juice and bronchoalveolar lavage fluid must be used. The sensitivity of these methods varies and depends upon the collection of sufficient quality and quantity of specimens, proper preparation of specimens, good staining technique, careful observation, and availability of a good microscope. Hence, the need to improve its operational capacity by way of research and development. Such tests often cannot be performed on all specimens in developing countries. It is thus important to improve the operational practicability of this method through research and development.

Smear microscopy is inconvenient for patients because they must submit sputum one by one to a medical facility for two or three days, when their transportation to the medical facility is not convenient. Therefore, some of the patients drop out during sputum collection phase. It has been shown conclusively that high-quality microscopy of two consecutive sputum specimens identifies the vast majority (98%) of smear-positive TB patients. WHO policy on case detection via microscopy has been revised to recommend a "same-day diagnosis" approach (microscopy of two consecutive sputum specimens on the same day) on patients and public health [10].

Fluorescence microscopy is more rapid and sensitive. However, it requires a stable power supply, greater expertise in reading and microscopy and interpretation of the finding, so not all samples cannot be tested with fluorescence microscopy in developed countries. Specimen culturing is required to isolate the M.TB strain to do drug susceptibility test. Recently, in developed countries, the prevalence of infections with non-tuberculous mycobacteria (NTM) infection has been increasing. Even if many medical resources are available, it is impossible to distinguish between M.TB and NTM based on morphological differences in smear staining findings and it is difficult to identify NTM species using the existing nucleic acid identification method. Patients with NTM require standard treatment that is specific to each NTM species, so it is crucial to isolate the strain, identify it, and perform drug susceptibility tests.

M.TB is a slow-growing bacterium and requires 1–4 weeks for culturing in a liquid medium. The culture examination systems are much expensive and may be difficult to maintain in low-income developing countries because of certain requirements, such as the constant electrical power supply and the quality control and appropriate facility management. Testing methods, including culture and drug susceptibility test, must be reviewed in developing countries with TB high prevalence.

4.2 Advances from Microbiological Reference Standard

Previously, rapid diagnosis of TB depended on smear microscopic tests. Detection of mycobacteria in sputum smears have provided a strong basis for diagnosing pulmonary TB until recent years, but a definitive diagnosis requires a positive acid-fast bacillus culture or nucleic acid amplification tests (NAATs).

In 2021, WHO strongly recommended molecular assays intended as the initial tests for the diagnosis of pulmonary and extrapulmonary TB and rifampicin resistance in both symptomatic adults and children, in preference to the previously recommended microbiological reference standard [11].

Especially, WHO specifically recommended NAATs for patients seeking care at health care facilities and, in particular, Xpert MTB/RIF or Xpert MTB/RIF Ultra as an initial test for the diagnosis of pulmonary TB and rifampicin resistance [11]. NAATs can provide results within 24–48 h and confirm the presence of M. TB in 50–80% of AFB smear-negative, culture-positive specimens. These advantages can

affect patient care and TB control. Costs can be reduced by basing decisions on NAATs results.

TB control, the problem of drug-resistant TB, especially multidrug-resistant TB, is gradually increasing. Conventionally, drug susceptibility tests have been performed based on culture results, but the development of NAATs has made it possible to diagnose TB and detect rifampicin resistance at the same time. Diagnosis was directly linked to treatment, enabling rapid diagnosis in patients with rifampicin-resistant TB. Funds for TB control have installed Xpert systems in economically developing countries.

WHO recommended that NAATs, including Xpert.TB.RIF, may replace traditional sputum smear and culture tests for patients with suspected pulmonary TB, so that an accurate and latest diagnosis method of pulmonary TB is to use NAATs [11].

4.3 Chest Imaging

If pulmonary TB is suspected based on the patient's medical history and symptoms, diagnostic imaging should be done in parallel with the sputum TB examination. As a precaution to prevent airborne infection in the X-ray room, the patients with cough should wear a face mask. Also, care should be taken to ventilate the X-ray room to prevent nosocomial infections from occurring in the X-ray room. Chest X-ray is useful but each finding is not specific to active pulmonary TB. It must be emphasized that although chest imaging is useful for suggesting pulmonary TB, chest imaging alone cannot establish accurate diagnosis of pulmonary TB. Bacteriological confirmation should always be attempted.

4.3.1 Chest X-Ray Diagnosis

Primary TB lesions appear in any lung field. In primary TB, MTB is spread throughout the body via hematogenous or lymphatic vessels, and occasionally, abnormal shadows in the lungs may appear later. Primary TB causes miliary TB with swelling of hilar and mediastinal lymph nodes and miliary TB.

Secondary (post-primary) pulmonary TB lesions are common in the upper lobe and the posterior upper segment of the lower lobes (S6) of the lung, often accompanied by satellite lesions. In patients with mild pulmonary TB, chest X-rays usually show nodular shadow or cavitation on one side of the apex of the lung, with small granular shadows scattered around the lesion. Chest imaging findings are affected by the patient's immune status and may change significantly over time.

In secondary pulmonary TB, chest X-rays show many radiological features; e.g., centrilobular nodules, nodules with or without cavitation, small opacities, and consolidation. Image findings of healed lesions include fibrotic sclerosing lesions, calcified lesions, and pleural thickening, which are often mixed. When these fibrotic lesions are extensive, tracheal deviation, atelectasis, and diaphragmatic elevation

are found. It should be noted that atypical findings on radiographs of immunocompromised hosts may lead to misdiagnosis of M.TB infection.

4.3.2 Computed Tomography

Chest CT imaging can capture all the lung fields. Early detection of the minimum shadow is possible, especially with high-resolution CT(HRCT). HRCT findings in patients with active pulmonary TB include micronodules, tree-in-bud appearance, nodules, air-space consolidation, ground glass opacities and cavities.

Miliary TB, unlike normal TB, progresses to hematogenous or lymphatic vessels, and small granular shadows 1–3 mm in diameter that forms almost uniformly throughout the lung field. Miliary TB may be associated with pleural lesions and swelling of the hilar and mediastinal lymph nodes.

There are many diseases that should be distinguished from pulmonary TB. Those with infiltrative shadows include bacterial pneumonia, atypical pneumonia, lung cancer, sarcoidosis, eosinophilic pneumonia, pulmonary parasite disease, pulmonary infarction, and eosinophilic granulomatosis. Those with nodular shadows include lung cancer, benign tumors of the lung, pulmonary mycosis, pulmonary sequestration, and pulmonary arteriovenous fistula. Those with a hollow shadow include lung abscess, lung cancer, lung parasitosis, and pulmonary mycosis. Diseases showing scattered shadows include pulmonary malignancies (including metastases), pneumoconiosis, sarcoidosis, hypersensitivity pneumonitis, and alveolar proteinosis. Especially in the elderly, there are residual scars of past diseases and comorbid lung diseases such as COPD and interstitial pneumonia. Therefore, it may be difficult to determine if it is an active TB lesion, and care should be taken so as not to overlook active pulmonary TB.

4.3.3 Other Imaging

The resolution of magnetic resonance imaging (MRI) is slightly lower than that of CT due to the imaging time and respiratory problems, but it can even estimate fluid and fat composition and understand blood flow conditions. Qualitative diagnosis by MRI is useful when tubercle bacilli are not detected in sputum or when bronchoscopy cannot be performed.

4.4 Bronchoscopy

Bronchoscopic (or bronchofibrescopic, BF) examination helps to distinguish between various lung diseases such as lung cancer and pulmonary TB that does not present sputum even if pulmonary TB is suspected by diagnostic imaging. BF examination is useful, especially for the diagnosis of tracheal and bronchial

TB. However, BF examination induces coughing, which increases the risk of nosocomial infections if the patient has pulmonary TB. When patients with suspected pulmonary TB are unable to present induced sputum, BF test should be done with precautions against airborne infection. To prevent nosocomial infections, BF tests should be performed in a negative-pressure room, and all participants in the room should wear an N95 mask.

Brushing, bronchoalveolar lavage, and biopsy of the target lesions are performed. The bronchoalveolar lavage fluid (BALF) and saline used to clean equipment used for brushing and biopsy are tested as same as sputum examinations. The lung biopsy material should be immersed in saline before fixing to formalin. However, it is important to obtain MTB culture for drug susceptibility testing.

On smear examination, the sensitivity of induced sputum is 0.35 (95% CI, 0.29–0.42) and the specificity is 0.99 (95% CI, 0.96–1.00), with certain defined conditions as the gold standards. The sensitivity of BALF is 0.38 (95% CI, 0.32–0.45), the specificity is 0.99 (95% CI, 0.96–1.00). There is no statistically significant difference in AUC on the ROC curve ($p = 0.792$). In culture studies, induced sputum sensitivity is 0.72 (95% CI, 0.66–0.77) and specificity is 1.00 (95% CI, 0.99–1.000). The sensitivity of BALF is 0.70 (95% CI, 0.64–0.75) and the specificity is 1.00 (95% CI, 0.99–1.00). There is no statistically significant difference in AUC on the ROC curve ($p = 0.602$). This review shows that sputum induction should be used first because BF testing is expensive and sputum induction is safer [12].

However, BF examination may be required especially for PLWHIV. Few studies have examined the significance of bronchoscopy in HIV patients. According to the article in South Africa, MTB/RIF on the BALF of patients who were smear negative or sputum scarce had shown excellent performance for the detection of TB in an HIV-prevalent setting [13]. Thoracoscopic examination is useful for diagnosis in cases of pleural effusion and peripheral lesions of the lung. There is video-assisted thoracoscopic surgery (VATS) that can be performed under local anesthesia. Even resection of the lung can be performed. It is useful when differential diagnosis is difficult by ordinary pleural biopsy or transbronchial biopsy.

5 New Technologies

Many advances have been developed as new diagnostic techniques for TB. On the other hand, the need for a point-of-care test is being discussed for countries with limited medical resources, but so far the progress is limited. In clinical practice, a path from symptoms, clinical findings, and radiological imaging, to bacterial testing is still the basic flow of diagnosis. Chest X-rays have been re-recognized as a useful test for triage and are also recommended by WHO for TB screening.

In addition, many biomarkers are being investigated. If any marker is discovered that can identify pulmonary TB using specimens that can be easily collected from patients, costly and painstaking tests such as BF chest imaging would be minimized

and undetected people with pulmonary TB could be reduced. Further research is also needed to evaluate the new test methods.

5.1 Lipoarabinomannan (LAM)

A simple kit for detecting lipoarabinomannan antigen, which is a cell wall component of acid-fast bacilli contained in urine, has been approved by WHO and is used for the diagnosis of TB. However, the sensitivity of the kit that initially preceded it is not high. After that, the sensitivity specificity of a new urinary LAM antigen detection kit by the ultra-sensitive immunochromatography method using the silver salt amplification technology developed by Fujifilm was reported [14], which contributed to the reduction of the mortality rate of PLWHIV. Using the microbiological reference standard, the estimated sensitivity of FujiLAM is 70.4% (95% CI 53.0 to 83.1) and the estimated specificity of FujiLAM is 90.8% (86.0 to 94.4). However, its usefulness in HIV-negative patients is unknown.

5.2 Other Biomarkers

Many tests have been developed to assist or confirm the diagnosis, and many serodiagnoses have been developed and used in countries with a high prevalence of TB. However, the diagnostic accuracy was not high, and false-negative and false-positive problems arose, especially in TB endemic countries, adversely affecting TB control. Therefore, WHO recommended not to use serodiagnosis previously [15].

Currently, most of these biomarkers are associated with host immunity. Numerous studies showing the correlation of these biomarkers with various phases of TB have been published. Biomarkers are needed in order to develop a diagnostic method for PLWHIV and children, using less invasive non-sputum specimens.

Volatile organic compounds (VOCs) in exhaled breath contain metabolites of M.TB and oxidative stress substances released from infected hosts, which are expected to be biomarkers for active pulmonary TB. It will take some time to put it to practical use [16].

Cytokines and immunostimulatory markers of CD4 + T cells are being studied for the purpose of diagnosing pulmonary TB [17], but further research is required for practical use.

5.3 Computer-Aided Detection Software

Chest imaging, like bacterial testing, occupies an important position in the diagnosis of pulmonary TB. However, accurate diagnosis of chest imaging can be difficult even for the specialists. A computer-aided diagnostic (CAD) system can efficiently

produce chest radiographs at low cost, and the software can analyze these digital images instantly. CAD analyzes the morphology of the lungs, detects the clavicle, detects abnormal findings in the lung field, and compares the shape with database TB lesions to calculate the probability of pulmonary TB.

WHO made a new statement on TB screening in March 2021 [18]; CAD is being recommended for the first time as an alternative to human interpretation of digital chest X-ray (CXR) for screening and triage for TB. Its use should be limited to the interpretation of plain CXRs for pulmonary TB in individuals aged 15 years or older. According to WHO, in screening use case, CAD sensitivity is 0.90 to 0.92, specificity is 0.23–0.66, and the sensitivity of CXR with human reader is 0.82–0.93, the specificity is 0.14–0.63. In triage use case, CAD sensitivity is 0.90–0.91, specificity is 0.25–0.79, while the sensitivity of CXR with a human reader was 0.89–0.96, and specificity was 0.36–0.63.

There are few pulmonologists and radiologists skilled in diagnosing pulmonary TB in areas with limited medical resources, so CAD will greatly contribute to pulmonary TB diagnosis. However, CAD cannot doubt the possibility of other illnesses. Pulmonologists can diagnose multiple diseases at the same time with diagnostic imaging. In order not to overlook diseases other than pulmonary TB, it is necessary to improve the response of the CAD diagnostic database, and ultimately, the ability of a pulmonologist or radiologist.

5.4 PET

Diagnosis of inflammatory disease is usually made by clinical symptoms, blood tests, X-ray findings, etc., but it is often difficult to make an accurate diagnosis and grasp the condition. Nuclear medicine diagnosis is one of the tests performed at such times, and is generally useful for differential diagnosis through evaluation of inflammatory lesions and inflammatory activity.

In recent years, positron emission tomography (PET) imaging with 18F-2-fluoro-2-deoxyglucose (18F-FDG) (FDG-PET) has been applied to the diagnosis of various inflammations and infectious diseases, suggesting that it may have high diagnostic performance [19]. Diagnosis of inflammation and infectious diseases by FDG-PET is not yet covered by health insurance worldwide, but the current situation is introduced here to focus on the new possibilities of FDG-PET.

Non-invasive detection of glucose metabolism abnormalities in the body using 18F-FDG enables diagnosis of malignant tumors, heart diseases, and inflammatory diseases. When compared to other nuclear medicine images such as gallium scintigraphy, PET can show the distribution of FDG three-dimensionally. FDG-PET has been proven to be useful indeterminate therapeutic efficacy.

The weak point of PET performance is that the minimum spatial resolution is low, and so it is not possible to obtain a clear image as much as CT, and it takes about thirty minutes to get the whole-body imaging. In addition, the relationship between the accumulation part and each organ is not clear, and it is necessary to compare it with CT. PET/CT was developed, which can take PET and CT under the same conditions and obtain a fusion image. Standardized uptake values (SUV) are

frequently used to evaluate the degree of FDG accumulation. When the administered radioactivity is uniformly distributed in the body, and the SUV is set to 1 and the relative accumulation in the target tissue is calculated. The higher the value, the higher the glucose metabolism.

Many papers have shown that 18F-FDG PET is useful in distinguishing between benign and malignant lung lesions. Regarding SUVmax by FDP-PET examination of mycobacterial granulomas, the average value of SUVmax in pulmonary mycobacterial infection in which tubercle bacilli or MAC disease was identified was 2.5 or higher [20]. In active mycobacterial active nodules and cavities, their imaging had higher SUVmax than in the cases with non-active shadows, and that there was a correlation between CT imaging and SUVmax. However, no clear cutoff value for distinguishing between benign and malignant has been defined, and careful observation is required for clinical application.

6 Special Status

6.1 Elderly

Changes in immune function with aging are called "immunosenescence." Decreased function of CD4-positive T cells (decreased acquired immunity) is the most characteristic feature of immunosenescence. Respiratory tract infections are especially common in the elderly, but with aging, the ability to prevent infection in the respiratory tract is reduced, and the response to inflammation is also low. It causes special symptoms and test results in the elderly. In this regard, TB is similar, and pulmonary TB in the elderly presents with a different medical condition than in younger ones. Many older patients with active TB often do not exhibit classical clinical features such as cough, hemoptysis, fever, night sweats, and loss of body weight.

Many papers reported the atypical chest imaging in the elderly [21]. pulmonary TB lesions may be seen in the middle or lower lung fields rather than upper fields in the elderly. Their radiological features often present consolidation similar to bacterial pneumonia. Typical types of fibrous nodules, with or without cavitary lesions, are less common, and consolidation or nodules similar to lung cancer occur more often. Immunosenescence increases the frequency of miliary TB. Sometimes, the elderly with normal radiological findings complains of low-grade fever and malaise, and later miliary shadows may appear in the bilateral lungs. If the diagnosis is delayed, the respiratory status may deteriorate rapidly and lead to death.

Elderly may have various shadows on the chest as sequelae of past illnesses, and it is very difficult to determine if a shadow suggesting fibrotic lesions after healing of pulmonary TB represents actually an inactive lesion.

Age-related changes in the immune system can affect the results of immunological tests that diagnose TB infections such as TST and IGRA. "Indeterminate" results are likely to occur regarding IGRA in the elderly [22]. In the elderly, there are no fixed reports that the microbiological diagnosis of TB may be affected by age. In areas of high burden in the elderly, sputum tests to distinguish pulmonary TB should be performed, if the elderly are suspected of having a respiratory infection disease.

6.2 People Living with HIV(PLWHIV)

MTB is one of the most virulent pathogens that cause opportunistic infections associated with HIV infection. People who are found to be infected with HIV should always be evaluated for TB infection. Screening for TB is important, regardless of whether they have received or are receiving ART. Those who do not have current cough, fever, weight loss, or night sweats are unlikely to have active TB. WHO reported these four symptoms and chest imaging were useful in diagnosing active TB, but four-symptom screening rule has a slightly lower specificity than chest imaging [23]. For PLWHIV, if TB occurs before cell-mediated immunity declines, it presents with typical TB symptoms and radiological findings. As CD4-positive T lymphocytes decline, TB progresses rapidly, and M.TB spreads hematogenous, resulting in miliarly and extrapulmonary tuberculous lesions. Extrapulmonary TB lesions such as swelling of the hilar, mediastinal lymph nodes, and pleural effusion are more common than typical lung imaging findings [24]. In addition, diagnosis based on sputum smears is less sensitive and may delay diagnosis.

With PLWHIV, attention should also be paid to the recurrence of TB. Therefore, when HIV patients complain of poor health, doctors should check for TB rather than just paying attention to subjective symptoms such as coughing, fever, weight loss, and night sweats.

Currently, in the 15 countries reported by WHO [1], areas with high HIV-positive rates and areas with high TB burden overlap. WHO warns that there are omissions of coinfection of HIV and TB in the report of each country. Health insurance schemes, or Universal Health Coverage (UHC) system, in economically developed countries contribute to the early detection of HIV or TB, while in developping countries it is clear that the prevalence of both diseases is high, however, the inspection system themselves have not caught up. Development of quick and inexpensive inspection is desired.

7 Case Findings

7.1 Low Prevalence Areas

7.1.1 Symptomatic Visit

As mentioned above, countries where TB patients have been declining and the incidence of newly registered TB patients per 100,000 population is under 10, are often economically developed countries, and medical institutions always deal with various diseases other than TB. People may even forget that there is TB in their country. In these countries, it is possible to diagnose TB relatively easily if clinicians notice that the patients with symptomatic visit have respiratory infectious disease and order sputum test for pulmonary TB. Since the symptoms are not specific for causing suspicion of pulmonary TB, it is important not to forget pulmonary TB as one

of the differential diagnoses of respiratory infections. In most cases diagnostic procedure is prompt and easy, starting with medical consultation, followed by physical examination, chest X-ray, sputum smear and culture tests, and the addition of NAAT. It is also important to always perform drug susceptibility tests. However, patients without sputum should receive CT scan and bronchoscopy. If positive evidence of sputum test is not available, empirical treatment for TB may be started, but in that case, clinicians should ask opinion of specialist. CAD system may help to diagnose when the diagnosis is complicated.

7.1.2 Contact Investigations

When a new pulmonary TB patient is diagnosed, an investigation is conducted to see if there is any undiagnosed pulmonary TB patient or newly infected persons among the family members and other close contacts of the initially detected patient.

In the systematic review [25] of 108 studies from high-income settings, the prevalence of TB among contacts was 1.4% (95% CI 1.1–1.8%), and the prevalence of latent infection was 28.1% (95% CI 24.2–32.4%). There was substantial heterogeneity among published studies.

In developed countries with low TB prevalence, active contact investigation will be able to obtain the detection of infected individuals, treatment before onset, and prevention of TB transmission. The article from the US CDC [26] in 2005 also stressed the importance of contact investigation in developed countries. In that case, IGRA should be performed instead of tuberculin reaction. If IGRA test is positive, chest imaging test is performed, and if pulmonary TB is suspected, sputum test, and NAAT are performed.

7.1.3 Screening

Screening is recommended for groups with high TB incidence in countries with low TB prevalence. Screening is necessary in situations with social background, such as refugees, immigrants from countries with high TB burden, prisons, and the poor people of urban areas, and disease-related situations, such as people who have been exposed to silica in the past, and PLWHIV.

7.2 TB Endemic Areas

7.2.1 Countries with High TB Burden but Abundant Resources

There are several economically well-off countries having yet high TB burden. It is necessary for their national TB programme to address awareness-raising activities for TB, preventive measures against nosocomial infections in the health facilities,

and detecting TB patients based on active epidemiological studies to prevent the spread of infection. At the same time, it is necessary to educate clinicians to prevent health system delay in TB diagnosis. Chest imaging, sputum smear culture test, NAAT, and drug susceptibility test must be performed as rapid and accurate TB diagnostic algorithms.

7.2.2 Countries with High TB Burden and Scarce Resources

In countries with limited resources, efficient patient detection should be done, instead of performing costly tests on all suspected patients, as WHO has shown. In the UN high-level meeting on TB ~~in~~ 2018, WHO stated to strengthen the policy so that 40 million people be treated for TB from 2018 to 2022 [1]. But for many of low- or moderate-income countries with high TB prevalence, implementation of systematic contacts investigation is not financially affordable. However, waiting for the patients who have become symptomatic is not enough to achieve the above ambitious goals. Therefore, the active case detection should be performed, i.e., triage based on symptoms and contact history, using chest images assisted by CAD. After that, NAAT is performed for the selected and suspicious individuals, to diagnose quickly and accurately.

8 Conclusion

NAAT is the latest test for diagnosing pulmonary TB. Xpert MTB/RIF and Xpert MTB/RIF ultra are the most useful tests that can diagnose RFP resistance at the same time. However, the latest approach to diagnosing pulmonary TB is to listen carefully to the patient's symptoms and suspect pulmonary TB. It has not changed in the past, but it is always the most basic and important thing.

References

1. World Health Organization. Global TB report 2020. 2020. https://apps.who.int/iris/bitstream/handle/10665/336069/9789240013131. Accessed 10 May 2020.
2. Conradie F, Diacon AH, Ngubane N, Howell P, Everitt D, Crook AM, et al. Nix-TB trial team. Treatment of highly drug-resistant pulmonary tuberculosis. N Engl J Med. 2020;382:893–902.
3. Miller LG, Asch SM, Yu EI, et al. A population-based survey of TB symptoms: how atypical are atypical presentations? CID. 2000;2000(30):293–9.
4. World Health Organization. Systematic screening for active TB Principles and recommendations. 2013. https://www.who.int/tb/publications/Final_TB_Screening_guidelines.pdf. Accessed 21 May 2021.
5. Field SK, Escalante P, Fisher DA, Ireland B, Irwin RS. And CHEST expert cough panel, cough due to TB and other chronic infections, CHEST guideline and expert panel report. Chest. 2018;153:467–97.

6. World Health Organization. Diagnostic and treatment delay in TB. 2009. https://apps.who.int/iris/bitstream/handle/10665/116501/dsa710.pdf?sequence=1&isAllowed=y. Accessed 21 May 2021.
7. Cai J, Wang X, Ma A, Wang Q, Han X, Li Y. Factors associated with patient and provider delays for TB diagnosis and treatment in Asia: a systematic review and meta-analysis. PLoS One. 2015;10(3):e0120088. https://doi.org/10.1371/journal.pone.0120088.
8. Quattrocchi A, Barchitta M, Nobile CGA, Prato R, Sotgiu G, Alessandra Casuccio A, Vitale F, Agodi A. CCM 2013 TB network. Determinants of patient and health system delay among Italian and foreignborn patients with PULMONARY TB: a multicentre cross-sectional study. BMJ Open. 2018 https://doi.org/10.1136/bmjopen-2017-019673.
9. Sasaki Y, Yamagishi F, Suzuki K, Kuriyama T. Patient's delay and doctor's delay in the pulmonary tuberculosis. Kekkaku. 1996;71:303–9.
10. World Health Organization. Same-day diagnosis of TB by microscopy: WHO policy statement. 2011. http://www.who.int/iris/handle/10665/44603#sthash.gvkTkPOD.dpuf. Accessed 21 May 2021.
11. World Health Organization. Section 1. Molecular assays intended as initial tests for TB. In WHO consolidated guidelines on TB. Module 3: diagnosis–rapid diagnostics for TB detection. 2020. https://www.who.int/publications/i/item/who-consolidated-guidelines-on-tuberculosis-module-3-diagnosis-rapid-diagnostics-for-tuberculosis-detection. Accessed 21 May 2021.
12. Luo W, Lin Y, Li Z, Wang W, Shi Y. Comparison of sputum induction and bronchoscopy in diagnosis of sputum smear-negative pulmonary tuberculosis: a systemic review and meta-analysis. BMC Pulm Med. 2020; https://doi.org/10.1186/s12890-020-01192-w.
13. Theron G, Peter J, Meldau R, Khalfey H, Gina P, Matinyena B, Lenders L, Govender U, Satshedu M, Dheda K. Accuracy and impact of Xpert MTB/RIF for the diagnosis of smear-negative or sputum-scarce tuberculosis using bronchoalveolar lavage fluid. Thorax. 2013;68:1043–51.
14. Broger T, Sossen B, Toit E, Kerkhoff AD, Schtz C, Reipold EI, et al. A novel lipoarabinomannan point-of-care TB test for people with HIV: a diagnostic accuracy study. Lancet Infect Dis. 2019;19:852–61.
15. World Health Organization. Commercial serodiagnostic tests for diagnosis of TB. Policy statement. 2011. http://apps.who.int/iris/bitstream/10665/44652/1/9789241502054_eng.pdf. Accessed 20 May 2021.
16. Phillips M, Basa-Dalay V, Blais J, Bothamley G, Chaturvedi A, Modi KD, et al. Point-of-care breath test for biomarkers of active Pulmonary TB. Tuberculosis (Edinb). 2012;92:314–20.
17. Goletti D, Lee MR, Wang JY, Walter N, Ottenhoff THM. Update on TB biomarkers: from correlates of risk, to correlates of active disease and of cure from disease. Respirology. 2018;23:455–66.
18. World Health Organization. WHO consolidated guidelines on TB. Module 2: screening–systematic screening for TB disease. 2021. https://www.who.int/Publications/i/item/9789240022676. Accessed 24 May 2021.
19. Kung BT, Seraj SM, Zadeh MZ, Rojulpote C, Kothekar E, Ayubcha C, et al. An update on the role of 18F-FDG-PET/CT in major infectious and inflammatory diseases. Am J Nucl Med Mol Imaging. 2019;9:255–73.
20. Demura Y, Tsuchida T, Ishizaki T, Mizuno S, Totani Y, Ameshima S, Miyamori I, Sasaki M, Yonekura Y. 18F-FDG accumulation with PET for differentiation between benign and malignant lesions in the thorax. J Nucl Med. 2003;44:540–8.
21. Schaaf HS, Collins A, Bekker A, Davies PD. Tuberculosis at extremes of age. Respirology. 2010;15:747–63.
22. Tebruegge M, de Graaf H, Sukhtankar P, Elkington P, Marshall B, Schuster H, et al. Extremes of age are associated with indeterminate Quanti FERON-TB gold assay results. J Clin Microbiol. 2014;52:2694–7.
23. World Health Organization: Guidelines for intensified tuberculosis case-finding and isoniazid preventive therapy for people living with HIV in resource-constrained settings. 2011.

http://apps.who.int/iris/bitstream/handle/10665/44472/9789241500708_eng.pdf?sequence=1. Accessed 24 May 2021.
24. Jones BE, Young SM, Antoniskis D, Davidson PT, Kramer F, Barnes PF. Relationship of the manifestations of tuberculosis to CD4 cell counts in patients with human immunodeficiency virus infection. Am Rev Respir Dis. 1993;148:1292–7.
25. Fox GJ, Barry SE, Britton WJ, Marks GB. Contact investigation for tuberculosis: a systematic review and meta-analysis. Eur Respir J. 2013;41:140–56.
26. The National Tuberculosis Controllers Association and Centers for Disease Control and Prevention. Guidelines for the investigation of contacts of persons with infectious tuberculosis; recommendations from the National Tuberculosis Controllers Association and CDC. MMWR. 2005;54:1–48.

Chapter 6
HRCT Diagnosis of Pulmonary TB: Microlesions

Harumi Itoh

Abstract The morphological features of the pulmonary lobules obtained by analysis of lung specimens were applied to the HRCT diagnosis of pulmonary TB. Among the pulmonary TB lesions, the HRCT features of centrilobular and branching nodules, bronchial and bronchiole lesions connecting multiple centrilobular and branching nodules, and the tree-in-bud pattern were explained. In particular, with regard to the tree-in-bud pattern, the valuable work previously performed in Japan was evaluated from a contemporary perspective.

Keywords HRCT of the lung · Pulmonary TB · Centrilobular nodule · Tree-in-bud pattern

1 Introduction

Radiography-based diagnostic imaging of pulmonary TB has advanced from simple X-ray images, through X-ray tomographic images, and to high-resolution computed tomography (HRCT) images. This has been supported by analysis of lung specimens (so-called radiologic–pathologic correlation, RPC). As will be touched upon later, the RPC carried out previously was surprisingly high-level, even from a contemporary perspective [1, 2], even though the image quality of the radiographic images at that time did not even reach the level of sample analysis.

After the advent of pulmonary HRCT, it became possible to diagnose mild lesions. Thus, the ability to read normal images, not only in pulmonary TB, became necessary, to match the technological innovations in image creation. This involved a comparison between knowledge of the existing lung structure, based on sample analysis, and normal HRCT, in contrast with radiologic–anatomic correlation

H. Itoh (✉)
Department of Radiology, Fukui University, Fukui, Japan
e-mail: hitoh@u-fukui.ac.jp

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(RAC). RAC has emphasized the importance of the peripheral lung structure centered on the lung lobules, across various respiratory diseases.

HRCT interpretation for various respiratory diseases is being pursued from the standpoint of radiologic–anatomic–pathologic correlation (RAP-C), which integrates RAC and RPC. Accordingly, the importance of the macroscopic imaging of lung specimens is increasingly recognized from the standpoint of diagnostic imaging. Although this has been emphasized in the past [1, 2], publication of texts in color, including specimens that have been accumulated since that time has great significance [3]. In that sense, pulmonary TB could be a model disease for promoting RAP-C, given its imaging properties of forming high-contrast microlesions [4, 5, 6].

2 HRCT of Pulmonary Lobules and Pulmonary TB

A morphological overview of pulmonary lobules will be described using lung specimens. This is the basis for HRCT interpretation of pulmonary TB.

1. Lobules as building blocks of the lungs

   Lung specimens with carbon deposits reveal that the lung is made up of a collection of lobules (Fig. 6.1). Carbon deposits are distributed over a wide area inside the lung, accumulating in the centrilobular nodule as one site, in the middle region as well as hilar region, and on the pleural side. This strongly suggests that the entire lung is composed of lobules. The HRCT image of a lung with pulmonary TB is shown in Fig. 6.2. This figure illustrates that the centrilobular and branching nodules of lungs with pulmonary TB are formed on the pleural side of the lung, in the middle region, and near the hilum.
2. Bronchioles as the skeletal framework of lobules

   The lobules form the periphery of the bronchi, and branch out in a centimeter pattern, while the bronchiole that branches out in millimeter pattern forms the skeletal framework [7] (Figs. 6.3, 6.4, and 6.5). The lobules are about 10 mm in size, and one lobule contains an average of 5 acini [7]. At the borders of the lobules, there are pulmonary veins, septa, pleura, interlobular bronchi, etc., while the lung parenchyma fills the space between the centrilobular region and the lobular boundary (Fig. 6.6). The ends of the bronchioles are the terminal bronchioles and respiratory bronchioles, and the centrilobular nodule (proximal lobule region) that includes these bronchioles and the surrounding alveolar region is at a distance of 5 mm or less from the border of the lobules and in the inner region of the lobules [5, 6] (Fig. 6.6).

   Normal bronchioles distributed in the centrilobular region cannot be imaged by HRCT. Instead, the normal pulmonary artery that runs parallel to the bronchioles can be confirmed by HRCT (Fig. 6.7). Knowing the rendering limits of the imaging system for pulmonary arteries in normal cases is the key to the success or failure of interpreting HRCT images of pulmonary TB. The pulmonary artery

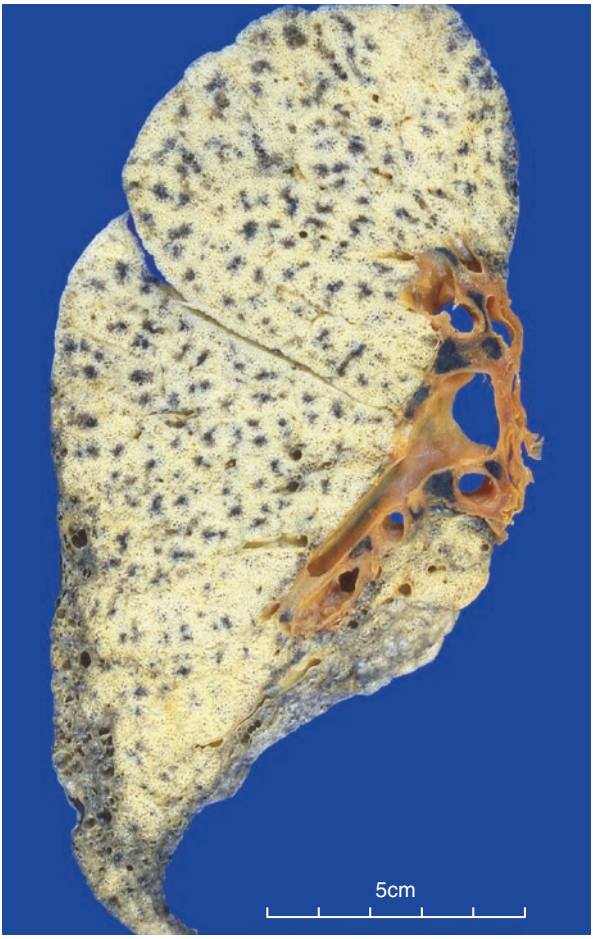

Fig. 6.1 Right lung specimen (coronal section). Black, small granular to bifurcated carbon deposits are observed on the pleural side of the lung, in the middle region, and near the hilum. The deposition site is a centrilobular area, and it does not come into contact with the pulmonary pleura, bronchi, pulmonary blood vessels, etc. In addition, there is normal lung tissue between adjacent deposit sites. In this case, chronic interstitial pneumonia is observed under the pleura. Centrilobular and branching nodules of pulmonary TB form in the same area as the carbon deposits

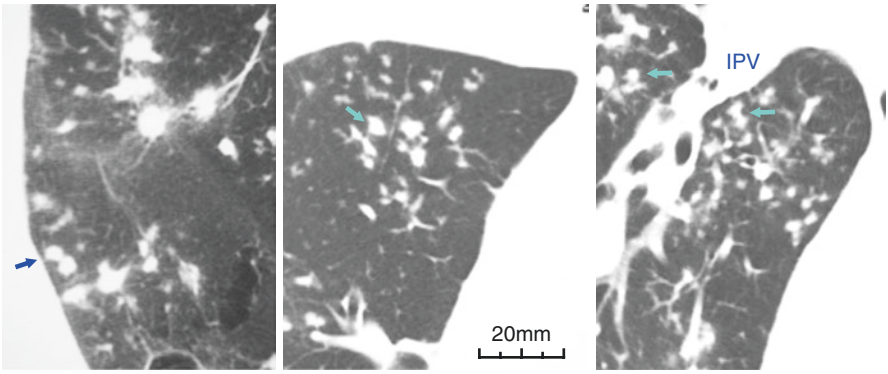

Fig. 6.2 High-resolution computed tomography of pulmonary TB. Centrilobular and branching nodules are observed in the subpleural region (left), middle area (center), and near the hilum (right). This clinical image indicates that the pulmonary lobules are widely distributed in the lungs. Each lesion is located a few millimeters away from the outer edge of the lung (where the pleura is located) and from pulmonary blood vessels (see arrow). IPV: lower pulmonary vein

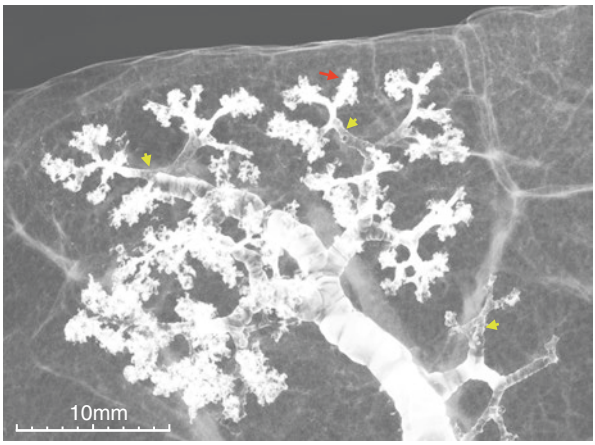

Fig. 6.3 Specimen bronchography. Barium solution was injected into the bronchi under fluoroscopy. The pleural side of the bronchi, the bronchioles, and some lung parenchyma are radiographed. At the periphery of the large bronchus, bronchioles with a diameter of 1 mm or less, which are characteristic of the intralobular airway, can be seen branching every few millimeters (the millimeter pattern) (yellow arrows). The tips of the bronchioles appear to bulge, like tree buds, because the contrast agent has flowed from the alveoli-bearing respiratory bronchioles to the center side of the alveolar ducts (red arrow)

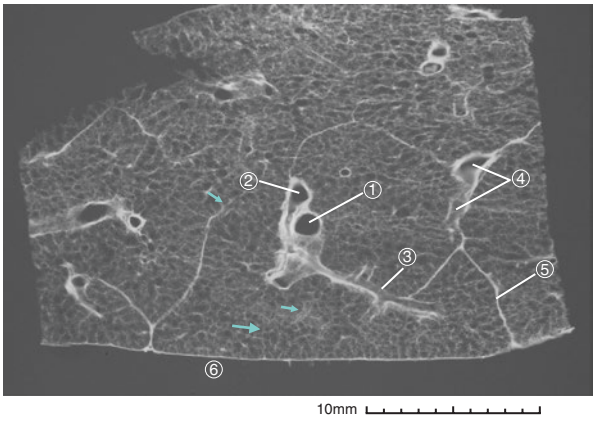

Fig. 6.4 Sample of an X-ray image of a sliced lung specimen. The specimen was taken from the pleural side of a normal area of the lower left lobe. It is one of 28 continuously sliced lung sections (thickness: 1 mm). The main lung structure that determines the extent of the lobules was identified over the entire slice. The (1) bronchus, (2) pulmonary artery, (3) bronchiole, (4) pulmonary vein, (5) lobular septum, and (6) pulmonary pleura are relatively easy to distinguish. The small pulmonary veins (small arrows) should be observed in detail and traced to the central side to be identified. The small septum (large arrow) is more difficult to see than the (5) lobular septum, but it is important for determining the lobular boundary. The branch of the (3) bronchiole shows a millimeter pattern

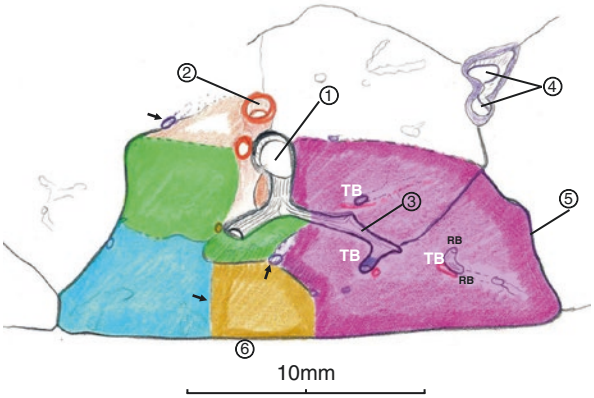

Fig. 6.5 An example of a lobular trace diagram. This represents a slice adjacent to that shown in Fig. 6.4. X-ray images and stereomicroscopic images of all sliced lungs were compared and examined to determine the range of each lobule. The structure and numbers are the same as in the previous figure. The flow of the alveolar tract, the small pulmonary veins, and small septa (arrows) were also noted. There is a terminal bronchiole (TB) at the tip of the bronchiole in the lobule marked with (3). The size of the lobule is close to 1 0 mm if intersected near the equator of the lobule (purple lobule)

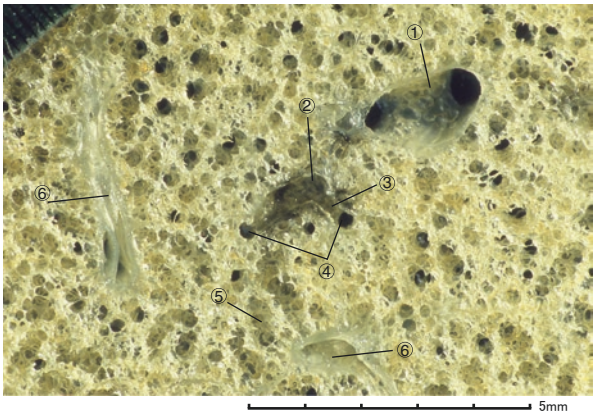

Fig. 6.6 Stereomicroscopic image of a centrilobular area. A normal image of the region where the centrilobular and branching nodule is formed. (1) Small bronchiole, (2) preterminal bronchiole, (3) terminal bronchiole, (4) first-order respiratory bronchiole, (5) alveolar duct, and (6) pulmonary vein. (1)–(4) are discrete and sparse tubular structures, but the (5) alveolar duct shows an air-rich, dense structure of various branches surrounded by alveoli, and is separated by the (6) pulmonary vein at the end of the lobule

that runs parallel to the terminal bronchiole has a diameter of 300–400 μ and is the smallest pulmonary blood vessel that can be rendered by HRCT (Fig. 6.7). On the other hand, the pulmonary artery that runs parallel to the respiratory bronchiole has a diameter of about 200 μ; current HRCT systems thus have

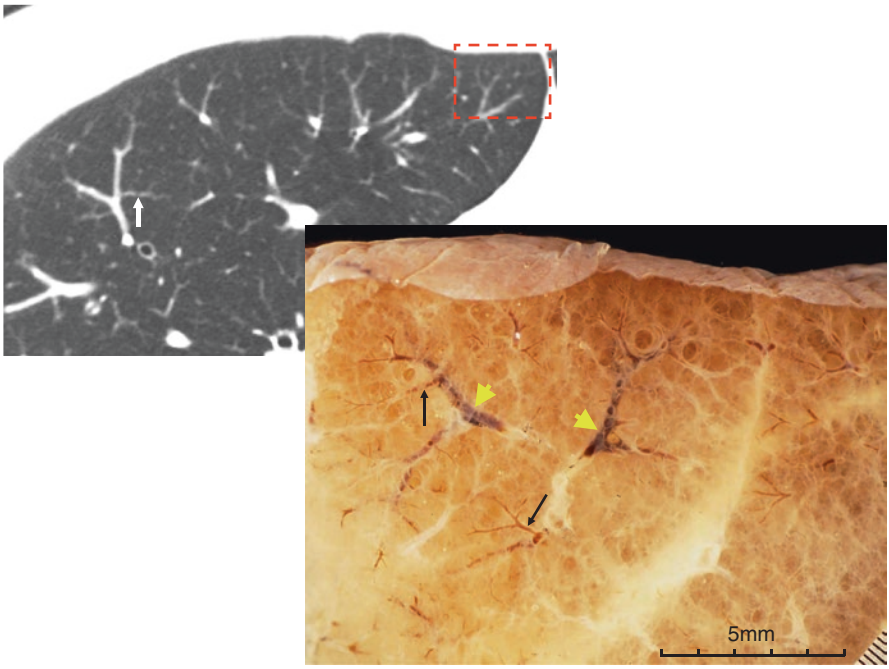

Fig. 6.7 Normal pulmonary artery visible on high-resolution computed tomography (HRCT). Comparison between normal HRCT image (upper left) and lung specimen (lower right). The intralobular pulmonary artery has main axis branches (lower right, thick arrows) that gradually decrease in diameter while bifurcating toward the lobular boundary, and thin side branches (lower right, thin arrows) that branch out from the main axis branch. In the HRCT image, the main axis branch (>300–400 μ diameter) until the centrilobular area is rendered, and the side branch cannot be shown (upper left, rectangular dotted frame). However, the thick side branch outside the lobule is visible (upper left, arrow)

difficulty in rendering the blood vessels below that level. Consequently, if a pulmonary blood vessel-like bifurcation shadow that exceeds the rendering limit is noted, it is regarded as an abnormal finding.

3. Centrilobular and branching nodules

   Similar to other infectious diseases, a centrilobular nodule is known to occur frequently in pulmonary TB. Centrilobular and branching nodules formed in pulmonary TB are high-contrast lesions of several millimeters in size [8] (Fig. 6.2). Centrilobular and branching nodules are inflammatory lesions that involve the terminal bronchioles, respiratory bronchioles, as well as their surrounding alveoli. Even if such lesions are found in multiple locations in the lobules, they do not reach the lung structure in the lobule borders, such as the pulmonary pleura and large pulmonary blood vessels, and leave a small gap (Fig. 6.2). When the bronchioles that connect the lesions together and the surrounding tissues are inflamed, thick bifurcation abnormalities are observed (Figs. 6.8, and 6.9). These nodular shadows and the set of bronchial and bron-

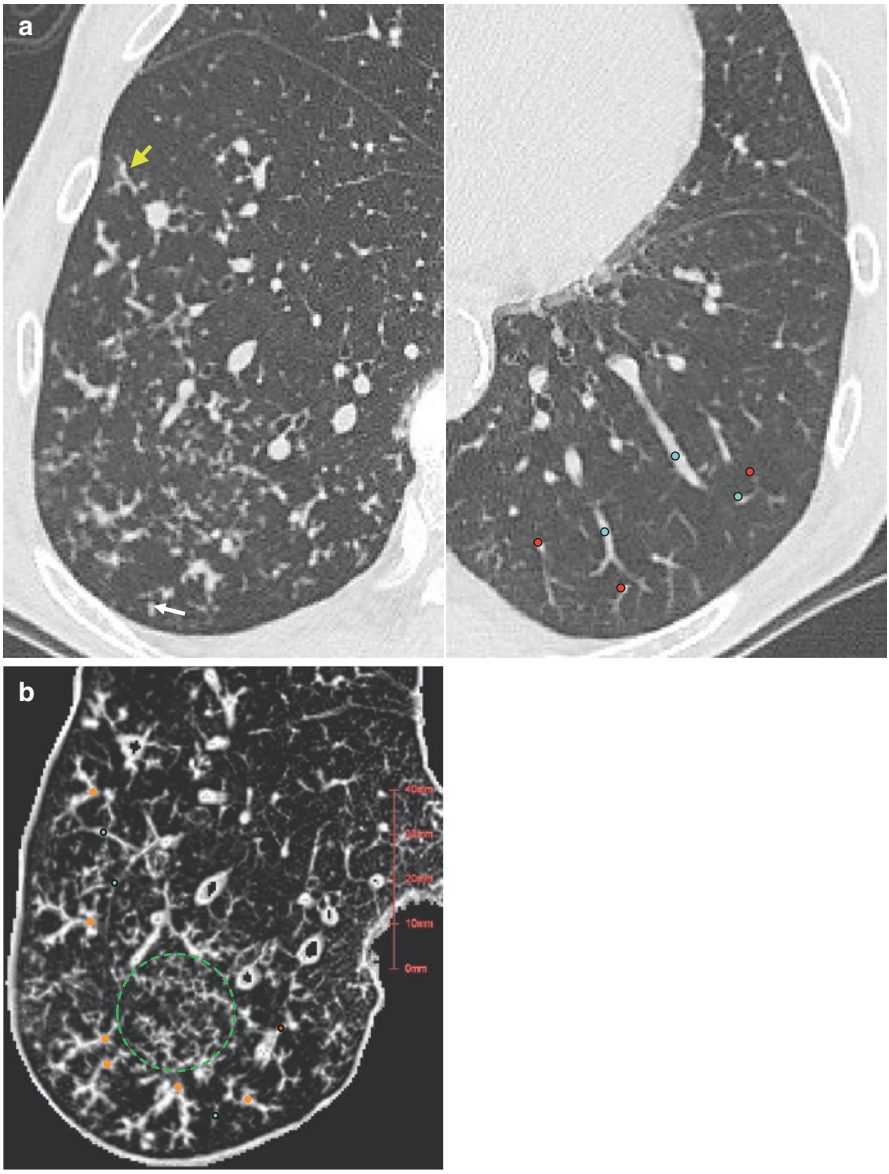

Fig. 6.8 High-resolution computed tomography (HRCT) image of pulmonary TB. 3D-computed tomography (right lung) in the same case as the conventional HRCT image (left and right lungs). In the left lung in Fig. 6.8a, pulmonary arteries (red circles) that can be regarded as almost normal can be observed. In comparison, in the lower right lung, thicker, bifurcated shadows with high absorbance can be observed up to the centrilobular nodule (thick arrow). There is also a centrilobular and branching nodule (thin arrow). In Fig. 6.8b, by continuously superimposing the HRCT images, abnormal bifurcated shadows extending inside and outside the lobule up to the centrilobular nodule are rendered (orange circles). There is no abnormality in the pulmonary veins (blue circles). In the image, there is an abnormal bifurcation shadow and the pulmonary artery is presumed to be split (red circle). A fine abnormal bifurcated shadow can be observed in the center, slightly to the caudal side of the figure (green dotted line frame: tree-in-bud pattern). Figure 6.8b is taken from Reference 6

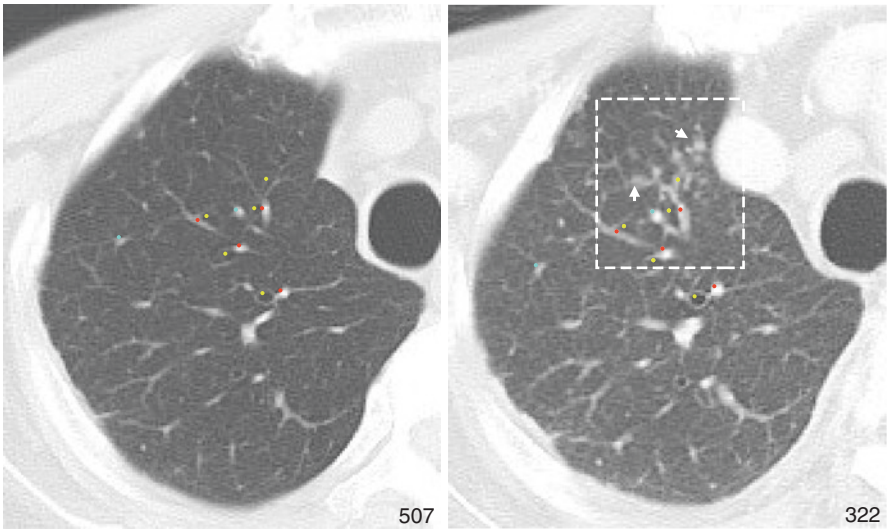

Fig. 6.9 High-resolution computed tomography (HRCT) image of pulmonary TB. HRCT image obtained at the time of the first consultation (left), and 16 months after the diagnosis of pulmonary TB was confirmed (right), showing changes in the upper right lobe in the same region. In the figure on the right, multiple centrilobular and branching nodules (arrows) and thickening of the central bronchial and pulmonary artery bundles connecting them are observed (dotted white rectangular frame on the right). By comparing the right figure with the normal computed tomography figure on the left, the formation and progression of the lesion can be understood. Yellow circles: bronchi; Red circles: pulmonary arteries; Blue circles: pulmonary veins

chiolar lesions can be interpreted on HRCT. However, in this process, it is important to confirm the normal image of the peripheral pulmonary artery (Figs. 6.8, and 6.9).

4. Tree-in-bud pattern

   Besides the centrilobular and branching nodules mentioned above, a group of finer lesions can also be observed, known as the tree-in-bud pattern [8], which has a pathology that is different from the centrilobular and branching nodules [6]. Lesions extend from the bronchioles in the lobules into the airspace of the respiratory bronchioles to the alveolar duct. Normally, the respiratory bronchioles and alveolar ducts are added to the size of the alveoli that open around them, with a width that is slightly larger than that of the terminal bronchiole on the center side (Figs. 6.3, 6.6, and 6.10). The tree-in-bud pattern is, in a sense, a state where the cavity is not filled with air, but rather with tuberculous exudate (Fig. 6.11). The “tree” in the “tree-in-bud” refers to strongly or weakly filled lesions along the long axis of the bronchioles inside and outside the lobules, while the “bud” refers to lesions extending from the respiratory bronchioles to the alveolar ducts [8]. Lesions may reach the lobular boundary (Figs. 6.11 and 6.12). In HRCT, the tree-in-bud pattern may be confused with the normal lung peripheral vascular shadow. To analyze the abnormal bifurcation shadow further,

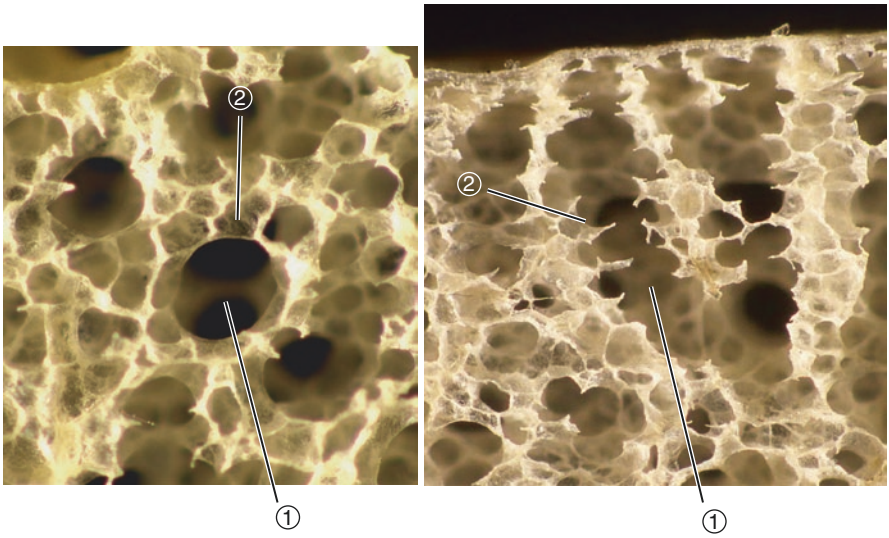

Fig. 6.10 Stereomicroscopic image of the normal alveolar duct. On the left is the short axis image, on the right is the long axis image. The alveoli (2) are open all around the proper air space (1) of the alveolar duct. When the lesion fills (1) and (2), the diameter is about 1 mm, and this becomes larger than the diameter from the preterminal to the terminal and first order respiratory bronchiole

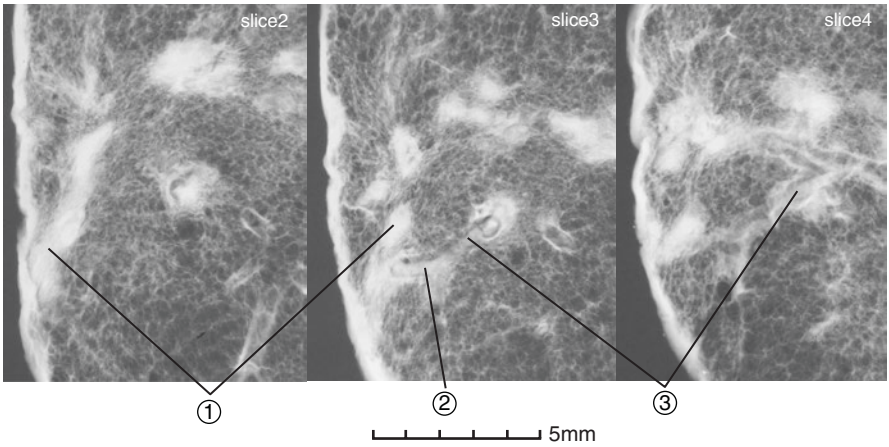

Fig. 6.11 Continuous X-ray images of a lung specimen showing the tree-in-bud pattern (3 figures). A dense, rod-shaped lesion (1) with a width of 1 mm or less, which reaches the pulmonary pleura and fills the alveolar duct and alveoli, continues from the lesion in the terminal bronchiole (2) to the lesion in the bronchiole on the central side (3), and is Y-shaped overall. Since the lung parenchyma around the lesion is almost normal, the contrast on the X-ray image of this lesion is high

the image can be read when the HRCT accuracy is high, and when there are no exudative issues in the lung around the lesion. This lesion shows extremely impressive histopathological findings (figure omitted, indicated in Reference 5).

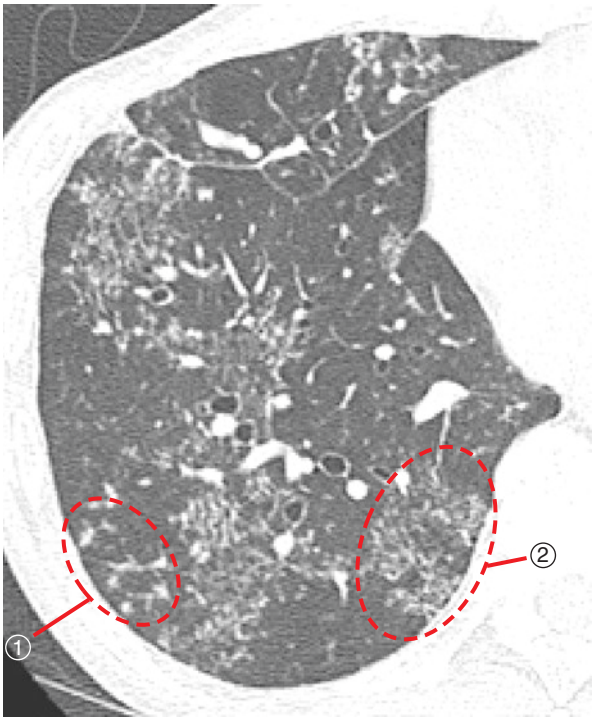

Fig. 6.12 High-resolution computed tomography (HRCT) image of pulmonary TB. This HRCT image has superior quality as compared to Fig. 6.8a, b. Thickening in the centrilobular and branching nodule and its central bronchial and pulmonary artery bundles are observed (dotted line frame (1)). In addition, micro-branched lesions (dotted line frame (2)) with different attributes can be observed. The latter is the tree-in-bud pattern, with the outermost part of the pattern reaching the pleura. Figure is taken from Reference 5

3 Achievements of Predecessors Who Influenced HRCT Diagnosis of Pulmonary TB

Japan has a history of pursuing diagnostic imaging of pulmonary TB in detail, based on RPC. One example is a case report of acinar pulmonary TB (referred to as the tree-in-bud pattern in this paper) [1, 2]. In 1939, a valuable report depicted an aerated lung obtained at an autopsy, which was expanded and fixed in a preserved state, and the X-ray image, trace diagram, and histologic image of the sliced lung were examined and compared with the chest X-ray images taken of the patient before death (Fig. 6.13). These images should be highly accurate and helpful, even when observed now.

The group image of microlesions distributed irregularly in Fig. 6.13 closely resembles the tree-in-bud pattern that can be observed on HRCT. The histology shows that the club-shaped lesion is swollen from the bronchioles to the alveolar ducts (Fig. 6.14). When the remaining trace diagram is scaled and the part pinpointed by the arrow is compared with the current HRCT image, although the state of image acquisition differed at the time, the image patterns are similar (Fig. 6.15). In both cases, the group image of bifurcated lesions with poor tapering that retains gaps can be observed.

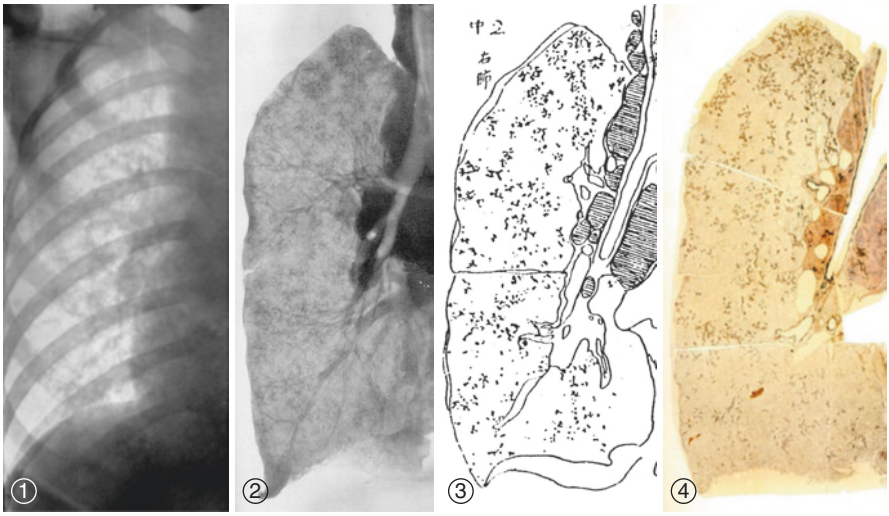

Fig. 6.13 Radiologic–pathologic correlation in diagnostic imaging of pulmonary TB (4 figures). From left to right, the chest X-ray image before death (1), X-ray image of inflated and fixed lung specimen slice (2), diffusely dispersed microlesions traced from the X-ray and macroscopic images (3), large intercept histopathological image (4). Minute Y-shaped lesions are captured in each of the images ((1) to (4)). It is a typical image of acinar TB. Images were taken from Reference 5

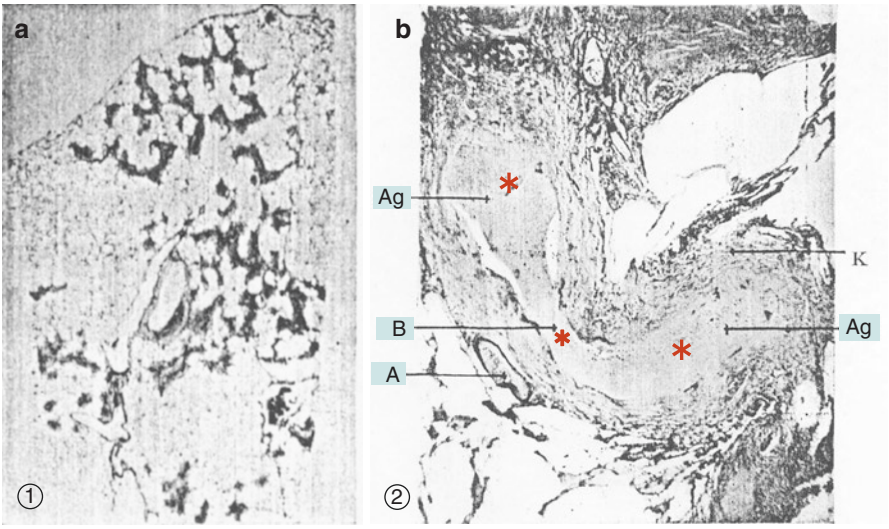

Fig. 6.14 A histopathological image of the same case as shown in Fig. 6.13. Micronodular to Y-shaped lesions can be observed in the weakly magnified image (1). In the strongly magnified image (2), the lesion* fills the alveolar duct (Ag) from the bronchiole (**b**) parallel to the pulmonary artery (**a**). Endobronchial lesions were thinner than alveolar duct lesions

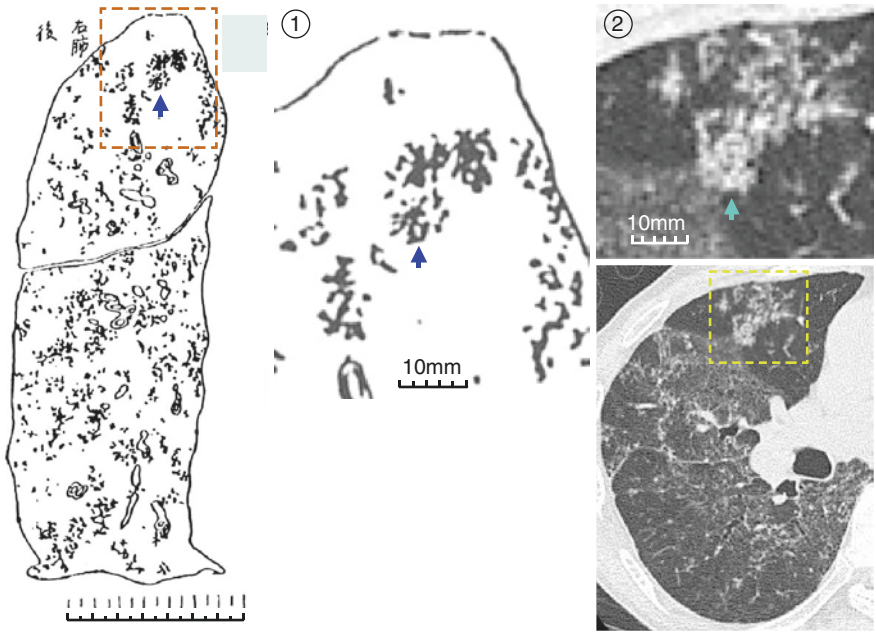

Fig. 6.15 Comparison of acinar pulmonary TB and tree-in-bud pattern in Fig. 6.13. This figure compares the trace diagram at the time (Center, (1)) and on high-resolution computed tomography 76 years thereafter (Right, (2)), on the same scale (arrow). The patterns in both images are very similar. Reference 1 states that these microlesions could not be rendered by conventional tomography at that time. Citation revised from Reference 5

References

1. Oka H, Hideo K. Pathological anatomy analysis of pulmonary tuberculosis X-ray images. First draft: proliferative acinar tuberculosis that closely resembles military tuberculosis. Practical. Med Sci. 1939;9(5):1–21.
2. Kumabe H. Pulmonary tuberculosis X-ray interpretation. Comparative study of pathological morphology and clinical practice. IV chronic pulmonary tuberculosis. Tokyo: Bunkodo; 1955.
3. Iwai K. Illustrative text of tuberculosis pathology. Illustrated/pathology of tuberculosis. Onset, progression, and mechanism of the aggravation of tuberculosis. Tokyo: Japan Anti-Tuberculosis Association; 2012.
4. Itoh H. Pulmonary tuberculosis imaging: a valuable educational resource for respiratory diagnostic imaging. Tuberculosis. 2010;85(12):869–79.
5. Itoh H. Pulmonary tuberculosis imaging diagnosis: radiologic–anatomic–pathologic correlation. Tuberculosis. 2016;91(11–12):667–76.
6. Itoh H. Pulmonary tuberculosis imaging: respiratory image basics. Tuberculosis. 2018;93(11–12):543–51.
7. Reid L. The secondary lobule in the adult human lung with special reference to its appearance in bronchograms. Thorax. 1953;13(2):110–5.
8. Im JG, Ito H, Shim YS, et al. Pulmonary tuberculosis; CT findings–early active disease and sequential change with antituberculous therapy. Radiology. 1993;186(3):653–60.

Chapter 7
Advances in Treatment of Drug-Resistant Pulmonary TB: What Is the Latest Approach to Treat Drug-Resistant Pulmonary TB?

Charles L. Daley

Abstract Drug-resistant strains of *Mycobacterium tuberculosis* continue to pose a major threat to global TB control. Despite the availability of curative anti-TB therapy, inappropriate and inadequate treatment, as well as unchecked transmission, has allowed drug-resistant strains of *M. tuberculosis* to spread globally. The World Health Organization (WHO) estimated that there were 465,000 cases of multidrug-resistant tuberculosis (MDR-TB)/rifampin-resistance tuberculosis (RR-TB) in 2019 and only 44% were detected and notified. Treatment of MDR-TB is challenging because it has traditionally required the administration of multiple anti-TB drugs, many associated with significant adverse reactions, for a prolonged duration. Globally, treatment outcomes remain poor with low treatment success and relatively high rates of treatment failure and lost to follow-up. Recently, there have been major advances in the treatment of MDR/RR-TB such as the availability of all oral regimens and shorter course regimens with high treatment success. This chapter will review the current approach to treatment of drug-resistant TB.

Keywords TB · Drug resistance · MDR-TB · Second-line drugs

1 Introduction

Drug-resistant strains of *Mycobacterium tuberculosis* continue to pose a major threat to global TB control. Despite the availability of curative anti-TB therapy, inappropriate and inadequate treatment, as well as unchecked transmission, has allowed drug-resistant strains of *M. tuberculosis* to spread globally. The World

C. L. Daley (✉)
National Jewish Health, Denver, CO, USA
University of Colorado, School of Medicine, Aurora, CO, USA
e-mail: daleyc@njhealth.org

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Health Organization (WHO) estimated that there were 465,000 cases of multidrug-resistant tuberculosis (MDR-TB)/rifampin-resistant tuberculosis (RR-TB) in 2019 of which only 206,030 (44%) were detected and notified [1]. In 2020, the number of notified cases of MDR/RR-TB dropped by 22% due to the impact of COVID-19 on health care systems [2].

Treatment of MDR-TB is challenging because it has traditionally required the administration of multiple anti-TB drugs, many associated with significant adverse reactions, for a prolonged duration. In 2020, 150,359 (95%) people with notified MDR/RR-TB were enrolled on treatment which is 15% less than in 2019 [2]. Treatment outcomes at the global level remain suboptimal with treatment success achieved in 59% of treated patients, up from 57% in 2019 and 50% in 2012. Fortunately, there have been major advances in the treatment of MDR/RR-TB such as the availability of all oral regimens and shorter course regimens that will be reviewed in this chapter.

2 Definitions of MDR, Pre-XDR, and XDR-TB

The WHO recently revised the definitions of drug-resistant TB because the transition from longer injectable-containing regimens to shorter, all oral regimens made the previous definitions obsolete (Fig. 7.1). In 2006, the WHO defined MDR-TB as in vitro resistance to at least isoniazid and rifampicin [3]. Extensively drug-resistant TB (XDR-TB) was defined as MDR-TB with additional resistance to fluoroquinolones and at least one second-line injectable (amikacin, kanamycin, or capreomycin) [3]. There was no definition for pre-XDR-TB. In the revised definitions, MDR-TB is defined the same as in the 2006 definition. However, a new

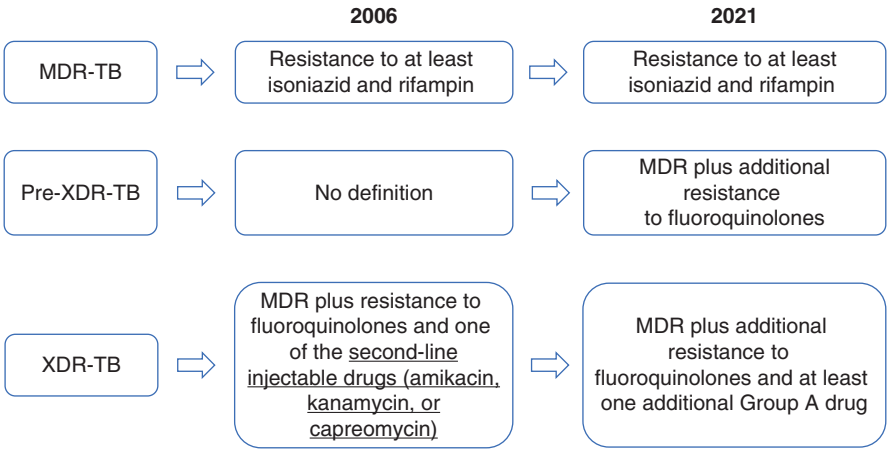

Fig. 7.1 Revised World Health Organization definitions for drug-resistant TB. Source: Meeting report of the WHO expert panel consultation on the definition of extensively drug-resistant tuberculosis. 27–29 October 2020. Geneva. World Health Organization, 2021. CC BY-NC-SA 3.0 IGO

pre-XDR-TB definition was added that is defined as MDR-TB plus additional resistance to fluoroquinolones. The definition of XDR-TB was modified to MDR-TB with additional resistance to fluoroquinolones and at least one additional Group A drug (bedaquiline or linezolid). Data supporting the new definitions come from a study that used an individual patient database meta-analysis that included over 11,000 patients with MDR-TB [4]. Investigators demonstrated that resistance to fluoroquinolones increased the odds of an unfavorable outcome (OR 1.91) and administration of bedaquiline and/or linezolid improved outcomes regardless of resistance to fluoroquinolones or second-line injectables. For individuals with XDR-TB, the odds of an unfavorable outcome were 0.37, 0.40, and 0.21 with use of linezolid only, bedaquiline only, and both, respectively [4].

3 Treatment of Drug-Resistant TB

3.1 Isoniazid-Resistant, Rifampin Susceptible TB

Isoniazid-resistant, rifampin-susceptible TB is a common form of resistant disease, and treatment outcomes are worse than with drug-susceptible disease. A systematic review examined the outcomes of INH-resistant TB compared with drug-susceptible TB and reported that treatment outcomes were poorer with higher treatment failure (11 vs. 2%), relapse (10 vs. 5%), and acquired multidrug resistance (8 vs. 1%) in those with resistant disease [5]. Both the WHO and American Thoracic Society (ATS)-led multi-society MDR-TB guidelines used data from a large individual patient meta-analysis that evaluated the treatment outcomes of 6 months of rifampicin, ethambutol, and pyrazinamide (with or without isoniazid) [6, 7]. Addition of a fluoroquinolone was associated with significantly better treatment success but with no significant effect on mortality or acquired rifampicin resistance. The WHO recommends continuing PZA throughout the course of therapy as the reduction in PZA to less than 3 months was associated with a worse treatment outcome, even with the addition of streptomycin [7]. The ATS states that in patients in whom toxicity from PZA is anticipated or experienced, or in those patients with low burden of disease, shortening the duration of PZA to 2 months may be considered [6].

3.2 MDR/RR-TB

Since the seminal publication by Iseman [8] in 1993, the approach to treating MDR-TB has usually included ≥4 drugs including a second-line injectable and administered for a long duration. The first WHO guideline on the management of MDR-TB was published in 1996 and adopted a similar approach to treatment but this approach has evolved over the years with the first recommendation for a shorter, standardized regimen in 2016 [9] and an all oral, shorter regimen in 2020 [7]. With

this evolution from longer to shorter regimens came the need to revisit our approach to drug selection and resulted in a new regrouping of second-line drugs by the WHO and multi-society guideline led by the ATS [6, 7]. Second-line drug dosages and associated adverse reactions are presented in Table 7.1.

Table 7.1 Drug doses for treatment of adults with MDR/RR-TB

Drugs	Route	Adult dose	Dose adjustment for renal disease	Dose adjustment for hepatic disease	Adverse reactions
Group A					
Bedaquiline	Oral	400 mg daily × 14 days then 200 mg 3 times/week	No change	No adjustment in mild to moderate disease	QTc prolongation, nausea/vomiting, arthralgia/myalga
Levofloxacin	Oral/ IV	750–1000 mg daily	3 times/week	No adjustment in mild to moderate disease	Abdominal distention, flatulence, diarrhea, arthralgia/myalgia, tendonitis, QTc prolongation, depression, psychosis, suicidal ideation, seizures, peripheral neuropathy, phototoxicity, ototoxicity, metallic taste
Moxifloxacin	Oral/ IV	400 (to 600–800) mg daily	No change	No adjustment in mild to moderate disease	Abdominal distention, flatulence, diarrhea, arthralgia/myalgia, tendonitis, QTc prolongation, depression, psychosis, suicidal ideation, seizures, peripheral neuropathy, phototoxicity, ototoxicity, metallic taste
Linezolid	Oral/ IV	600 mg daily	No change	No adjustment in mild to moderate	Cytopenias, peripheral neuropathy, optic neuritis, lactic acidosis, ototoxicity, alopecia

Table 7.1 (continued)

Drugs	Route	Adult dose	Dose adjustment for renal disease	Dose adjustment for hepatic disease	Adverse reactions
Group B					
Clofazimine	Oral	100 mg daily	No change	Use with caution in severe disease	Skin discoloration and dryness, phototoxicity, nausea/vomiting, hepatitis, QTc prolongation
Cycloserine	Oral	250–750 mg daily to achieve serum concentration of 20–35 mg/l	250 mg daily and adjust based on serum drug concentration	No change	Depression, psychosis, suicidal ideation, seizures, peripheral neuropathy, ototoxicity
Terizidone	Oral	250–750 mg daily to achieve serum concentration of 20–35 mg/l	250 mg daily and adjust based on serum drug concentration	No change	Depression, psychosis, suicidal ideation, seizures, peripheral neuropathy, ototoxicity
Group C					
Ethambutol	Oral/ IV	15–25 mg/kg daily	3 times/week	No change	Peripheral neuropathy, optic neuritis, nausea/ vomiting, arthralgis/ myalgia
Delamanid	Oral	100 mg twice daily	Mild to moderate renal insufficiency—no change	Not recommended in moderate to severe disease	QTc prolongation, nausea/vomiting
Pyrazinamide	Oral	25–40 mg/kg daily	3 times/week	Use with caution	Hepatitis, phototoxicity, arthralgia/myalgia
Imipenem and cilastin	IV	1000 mg 3–4 times/day	May reduce frequency	No change	Cytopenia, rash, nausea/vomiting, seizures
Meropenem	IV	1000 mg 3 times/day	May reduce frequency	No change	Cytopenia, rash, nausea/vomiting, seizures

(continued)

Table 7.1 (continued)

Drugs	Route	Adult dose	Dose adjustment for renal disease	Dose adjustment for hepatic disease	Adverse reactions
Clavulanate (used with carbapenems)	Oral/ IV	250 mg 3 times/day	May reduce frequency	No change	Side effects of amoxicillin/ clavulanate: nausea/ vomiting, flatulence, diarrhea, headache, rash
Amikacin	IM/ IV	15 mg/kg daily 25 mg/kg 3 times/week	15 mg/kg 2–3 times/week	No change	Peripheral neuropathy, ototoxicity, nephrotoxicity
Streptomycin	IM/ IV	15 mg/kg daily 25 mg/kg 3 times/week	15 mg/kg 2–3 times/week	No change	Peripheral neuropathy, ototocity, nephrotoxicity
Ethionamide	Oral	15–20 mg/kg	No change	Use with caution	Depression, suicidal ideation, peripheral neuropathy, optic neuritis, nausea/ vomiting, abdominal distention, flatulence, diarrhea, hepatitis, ototoxicity, arthralgia/myalgia, alopecia, metallic taste, hypothyroidism, gynecomastia
Prothionamide	Oral	15–20 mg/kg	No change	Use with caution	Depression, suicidal ideation, peripheral neuropathy, optic neuritis, nausea/ vomiting, abdominal distention, flatulence, diarrhea, hepatitis, ototoxicity, arthralgia/myalgia, alopecia, metallic taste, hypothyroidism, gynecomastia
Para-aminosalicylic acid	Oral	4 g 2–3 times/ day	No change	Use with caution	Nausea/vomiting, abdominal distention, flatulence, diarrhea, hepatitis, hypothyroidism

Table 7.1 (continued)

Drugs	Route	Adult dose	Dose adjustment for renal disease	Dose adjustment for hepatic disease	Adverse reactions
Other drugs					
Pretomanid	Oral	200 mg daily	Insufficient data	Insufficient data	Insufficient data. Used in combination with bedaquiline and linezolid
High-dose INH	Oral/ IV	15 mg/kg daily	No change	Use with caution	Hepatitis, nausea/ vomiting, peripheral neuropathy

Sources: Lange C, et al. Management of drug-resistant tuberculosis. Lancet 2019;394:953–66. Nahid P, et al. Treatment of drug-resistant tuberculosis. An official ATS/CDC/ERS/IDSA clinical practice guideline. Am J Respir Crit Care Med 2019;200:e93–e142

Table 7.2 Grouping of second-line drugs by World Health Organization (WHO) and multi-society[a] MDR-TB guidelines

WHO	Drugs	ATS/CDC/ERS/IDSA[a]
Group A	Levofloxacin or moxifloxacin Bedaquiline	*Strong recommendation for*
	Linezolid	*Conditional recommendation for*
Group B	Clofazimine Cycloserine or terizidone	
Group C	Ethambutol Delamanid Pyrazinamide Carbapenems with clavulanate Amikacin or streptomycin	
	Ethionamide or prothionamide P-aminosalicylic acid	*Conditional recommendation against*
Do not use	Kanamycin Capreomycin	
	Macrolides Amoxicillin/Clavulanate	*Strong recommendation against*

[a]*ATS* American Thoracic Society, *CDC* Centers for Disease Control and Prevention, *ERS* European Respiratory Society, *IDSA* Infectious Diseases Society of America
Sources: Nahid P, et al. Treatment of drug-resistant tuberculosis. An official ATS/CDC/ERS/IDSA clinical practice guideline. Am J Respir Crit Care Med 2019;200:e93–e142. World Health Organization. Module 4. Treatment of drug-resistant tuberculosis

3.2.1 Grouping of Second-Line TB Drugs

The WHO and ATS-led multi-society guidelines have grouped the second-line drugs based on current evidence of their effectiveness and safety as identified in a large individual patient meta-analysis [6, 7] (Table 7.2). The WHO divides second-line drugs into three groups (A–C) with drugs listed in order of general preference

[7]. Group A includes the later generation fluoroquinolones, bedaquiline, and linezolid. Group B includes clofazimine and cycloserine (terizidone). Group C includes ethambutol, delamanid, pyrazinamide, carbapenems, and amikacin (streptomycin). The WHO recommends against the use of capreomycin, kanamycin, ethionamide/prothionamide, macrolides, and amoxicillin/clavulanate.

The ATS-led multi-society guidelines follow a similar approach as they used the same individual patient meta-analysis but weighed the information slightly differently in three areas (Table 7.2) [6]. First, the guideline has a strong recommendation for the use of levofloxacin or moxifloxacin and bedaquiline but moved linezolid into those drugs for which a conditional recommendation is provided. Second, this category includes all of the drugs in the WHO Group C except the ATS provides a conditional recommendation against the use of ethionamide (prothionamide) and PAS as well as kanamycin and capreomycin. And finally, ATS makes a strong recommendation against the use of macrolides and amoxicillin/clavulanate.

Group A Drugs

Fluoroquinolones (Moxifloxacin and Levofloxacin)

Fluoroquinolones inhibit the enzyme DNA gyrase in *M. tuberculosis* which blocks the supercoiling of DNA. Fluoroquinolones have bactericidal and sterilizing activity, are well tolerated, and resistance to these drugs is associated with higher rates of treatment failure and death [10, 11] whereas inclusion of fluoroquinolones in the treatment regimen is associated with improved outcomes [12, 13]. Resistance occurs due to mutations in the genes *gyrA* and *gyrB* but the degree of resistance varies depending on which fluoroquinolone and concentration are used to define resistance [14, 15]. Zignol et al. [16] reported that among 282 strains of *M. tuberculosis* deemed resistant to ofloxacin at 2.0 μg/ml, 72% were resistant to moxifloxacin at 0.5 μg/ml, but only 7% were resistant to moxifloxacin and 2% to gatifloxacin at a concentration of 2.0 μg/ml.

Early bactericidal activity (EBA) studies demonstrated that levofloxacin has high EBA, equivalent to moxifloxacin, when given at high doses (1000 mg/day) [17, 18]. In a retrospective study from South Korea that included 171 patients, treatment success was similar between levofloxacin and moxifloxacin although only 48 patients received the latter drug (normal dose) and the patients that received moxifloxacin had isolates with resistance to significantly more drugs and higher incidence of ofloxacin resistant than those who took levofloxacin (78.9 vs. 83.3%, $p = 0.42$) [19].

Bedaquiline

Bedaquiline (TMC-207) is a diarylquinoline that inhibits mycobacterial ATP synthase [20] and has good but delayed bactericidal and sterilizing activity [11]. Mutations in the *atp*E gene are associated with high-level resistance and result in

cross-resistance with clofazimine. The drug is administered with food as bioavailability is doubled with a high-fat meal. Bedaquiline is metabolized by CYP3A4 so plasma concentrations can be significantly decreased by induces of this enzyme system and increased by inhibitors.

In a randomized placebo-controlled study of 47 patients with MDR-TB, bedaquiline increased the proportion of patients with conversion (48 vs. 9%) [20] and reduced the time to culture conversion over 24 weeks of observation [21]. In addition, none of the patients who received bedaquiline acquired resistance to ofloxacin compared with 22% of those in the placebo-controlled arm. A subsequent phase 2, open-label, single-arm study reported that 72% of 233 MDR/XDR-TB patients converted cultures by 120 weeks and the bedaquiline-containing regimens were well tolerated [22]. A large retrospective study including 428 MDR-TB patients reported a 71.3% treatment success with interruption of therapy in only 5.8% of patients [23].

Linezolid

Linezolid is an oxazolidinone antibiotic with moderate bactericidal and possibly good sterilizing activity [11] that works by inhibiting ribosomal translation through binding to the 23S subunit. Resistance occurs through point mutations in the peptidyl transferase domain of the 23S ribosomal RNA.

A systematic review and meta-analysis that included 12 studies with 207 patients concluded that linezolid appeared to provide added benefit to multidrug regimens although drug-related toxicity was common [24]. Culture conversion was reported to have occurred in 100/107 (93.5%) receiving individualized linezolid-containing MDR-TB regimens. Eighty-two percent of the patients were treated successfully and there was no difference in outcomes when stratified by doses above or below 600 mg daily. Based on this review, the authors concluded that ≤600 mg per day (single or divided doses) was the best option currently. Another systematic review and meta-analysis that included 11 studies with 148 patients reported a pooled treatment success of 67.9% and again noted no significant difference in outcomes based on whether the patient received ≤600 mg or >600 mg per day [25].

Linezolid inhibits protein synthesis in human mitochondria resulting in frequent toxicities. In a systematic review and meta-analysis, adverse events occurred in almost 60% of patients receiving linezolid of which 68% were considered major AEs: including anemia (38%), peripheral neuropathy (46%), gastrointestinal disorders (17%), optic neuritis (13%), and thrombocytopenia (12%). The frequency of AEs was significantly higher when the dose of linezolid was greater than 600 mg daily. Similarly, in the review by Cox et al. [25] adverse reactions were common resulting in the discontinuation of linezolid in approximately 36% of patients. The proportion of adverse events necessitating treatment discontinuation varied by dose: 29.5% for ≤600 mg and 60.75% for >600 mg (p =0.05). In the BPaL regimen, approximately 80% of patients who took linezolid experienced peripheral neuropathy [26]. The serum trough concentration of linezolid has been associated with toxicity.

Group B Drugs

Clofazimine

Clofazimine is a fat-soluble semisynthetic riminophenazine dye that has good activity in vitro against *M. tuberculosis* and has been shown to be highly active in both acute and chronic mouse models [27]. One study reported that clofazimine had dose-dependent, sustained bactericidal activity against *M. tuberculosis* persisters in a mouse model of chronic TB [28]. The mechanism of action has been postulated to be disruption of electron transport and generation of reactive oxygen although other mechanisms have also been suggested. The terminal half-life is 70 days.

A systematic review and meta-analysis reported the results of the use of clofazimine in the treatment of drug-resistant TB [29]. The review included 12 studies with a total of 3489 patients. Treatment success ranged from 16.5% in a small retrospective study in India to 87.8% in a cohort study in Niger; the pooled estimate of treatment success was 62.0%. The most common side effects were gastrointestinal disturbances and skin pigmentation.

Two retrospectives observational studies compared treatment outcomes of clofazimine versus non-clofazimine containing regimens. The first report was from Shanghai, China, and reported that no significant difference was seen in treatment outcomes among the 44 patients that received clofazimine although they were more likely to have received a fluoroquinolone and an injectable previously [30]. None of the patients that received clofazimine experienced major side effects requiring discontinuation however, the dose was reduced in 20 patients. Adverse reactions occurred in 88% of the patients, which included darkening of the skin (39), gastrointestinal adverse reactions (21), ichthyosis (12), and dizziness (1). The largest series using clofazimine in the treatment of MDR-TB was published from Brazil [31]. In this retrospective study, treatment success was 60.9% and similar to those who received pyrazinamide (64.6%) and adverse reactions were relatively uncommon. Van Deun and colleagues [32, 33] reported excellent treatment outcomes in patients with MDR-TB that received a seven-drug regimen that included clofazimine. No adverse reactions were attributed to clofazimine in that study.

A single randomized controlled study assigned 105 patients with MDR-TB in China to receive either an individualized regimen with or without clofazimine [34]. The use of clofazimine was associated with cavity closure, accelerated sputum culture conversion, and higher treatment success (73.6 vs. 53.8%).

Cycloserine and Terizidone

Cycloserine and terizidone (two cycloserine molecules) competitively block the enzymes that incorporate alanine into an alanyl-alanine dipeptide, which is an essential component in the mycobacterial cell wall [35]. Cycloserine is an oral bacteriostatic drug [11]. Early studies using cycloserine monotherapy produced a rapid clinical response and when combined with INH, patients also improved but INH resistance emerged [36]. When combined with ethionamide, patients usually converted cultures to negative [37].

Group C Drugs

Pyrazinamide

PZA, a pro-drug activated by *pncA*, has potent sterilizing activity [11] and is used during the initial phase of anti-TB treatment with the goal of shortening therapy [35]. Resistance to PZA is conferred by several mutations including mutations in *pncA* and *rpsA* [38]. In studies describing the outcome of MDR and XDR-TB patients, inclusion of PZA in the regimen was associated with better treatment outcomes than when PZA was not included in the regimen [39, 40]. In a recent large meta-analysis supporting the 2011 WHO guidelines, inclusion of PZA in the treatment regimen was associated with improved outcomes although the added benefit was small [41]. In a subsequent analysis, PZA resistance was not associated with a significant decrease in treatment success with either the shorter course or conventional regimen [9].

Ethambutol

Ethambutol is one of the least active of the first-line drugs but prevents the emergence of resistance to companion agents [11]. The drug works by inhibiting mycobacterial cell wall arabinosyltransferases, leading to depletion of arabinogalactan and lipoarabinomannan. Resistance is conferred by mutations in *emb*B but this only accounts for 60% of resistance. Ethambutol resistance is common among MDR-TB patients having been reported to occur in as much as 50–60% of MDR-TB cases but this varies widely [42]. Reproducibility of DST is relatively poor and not currently recommended by the WHO [43].

Delamanid

Delamanid (OPC-67683) is a nitro-dihydro-imidazooxazole derivative that inhibits mycolic acid synthesis and releases reactive oxygen species [44]. The drug has good bactericidal and sterilizing activity with activity against both growing and dormant bacilli [11]. Resistance occurs with mutations in the genes encoding for F420-dependent nitro-reductases.

Delamanid was evaluated in a randomized placebo-controlled multinational clinical trial in which 481 patients received delamanid 100 mg twice daily, delamanid 200 mg twice daily, or placebo for 2 months in combination with an optimized background regimen [44]. Culture conversion at 2 months in a liquid culture system was more likely in patients who received delamanid 100 mg twice daily (45.4%, $p = 0.0008$) or delamanid 200 mg twice daily (41.9%, $p = 0.04$) than placebo. Adverse events were distributed relatively equally across the three groups except that QTc prolongation was more common in the delamanid group.

A randomized, double-blind, placebo-controlled, phase 3 trial enrolled 511 MDR-TB patients from seven countries [45]. Subjects received delamanid or placebo added to a WHO longer MDR regimen. Median time to culture conversion did

not differ between the groups and there was no difference in the frequency of serious adverse reactions [45].

Early results with delamanid in the programmatic setting have shown high culture conversion rates and low frequency of QTc intervals over 500 ms [46–48]. Results from compassionate use in resource-limited settings demonstrated culture conversion at 6 months in 80% of patients with QTc prolongation in only 3.8% [49]. Among 49 MDR/XDR-TB patients treated with a delamanid-containing regimen in Korea, 96% culture converted and 81.6% achieved treatment success.

Carbapenems with Clavulanic Acid

M. tuberculosis is resistant to β-lactam antibiotics due to a class A β-lactamase which hydrolyses penicillins and cephalosporins. Resistance may be overcome by inhibition of the β-lactamase by adding clavulanate or the use of an antibiotic that is not a substrate for it like the carbapenems. Ideally, both approaches can be used by combining a carbapenem (imipenem or meropenem) with amoxicillin-clavulanate (clavulanate alone is not available in the market) [11].

β-Lactam antibiotics such as amoxicillin plus clavulanate and the carbapenems (imipenem, meropenem) have been shown to have anti-TB activity that is enhanced in the presence of clavulanate [27]. The combination of clavulanate and imipenem or meropenem significantly improved survival in a murine model although they did not prevent bacterial growth as isoniazid did. In a small clinical study of ten patients with MDR-TB and multiple risk factors for poor treatment outcomes, 1.0 gm of imipenem given twice daily was associated with sputum conversion in 8/10 patients: 7 patients remained culture negative off therapy at the end of the study [50]. A case-control study of meropenem and clavulanate plus linezolid-containing MDR-TB regimens were reported to be associated with a smear conversion rate at 3 months of 87.5 vs. 56% ($p = 0.02$) in controls [51]. Moreover, recently there have been several publications showing the efficacy of imipenem, meropenem, and ertapenem in the treatment of MDR and XDR-TB [52–55]. Based on these studies, it appears that the carbapenems have significant activity against drug-resistant *M. tuberculosis*, however, the high costs, lack of availability of oral formulations, and variable market availability limit their usefulness globally.

Aminoglycosides and Polypeptides

The aminoglycosides (streptomycin, kanamycin, and amikacin) and polypeptide (capreomycin) [35] are bactericidal and act primarily extracellularly although some intracellular activity has been demonstrated [11, 56]. All of the injectables have similar levels of in vitro activity and similar adverse reaction profiles although capreomycin is more likely to cause electrolyte disturbances such as hypokalemia and hypomagnesemia [41, 57, 58]. Unfortunately, all of these agents are ototoxic and have been reported to produce hearing loss in up to 50% of MDR-TB patients who received them [59, 60]. Although cross-resistance among the agents is common our understanding of the cross-resistance between the injectables has evolved with an

improved understanding of the mutational causes of resistance [35, 61]. It appears that isolates acquiring resistance to streptomycin usually remain susceptible to kanamycin, amikacin, and capreomycin [61–64]. Isolates acquiring resistance to capreomycin are usually susceptible to kanamycin and amikacin. Most isolates that acquire resistance to amikacin are also resistant to both kanamycin and capreomycin. And isolates acquiring resistance to kanamycin show different levels of cross-resistance to amikacin and capreomycin.

Thioamides (Ethionamide and Prothionamide)

The thioamides are pro-drugs that are activated by mycobacterial monooxygenase EthA and target the same acyl reductase enzyme InhA as INH. The drugs inhibit mycolic acid biosynthesis and the molecular target of ethionamide/prothionamide is *inhA* [42]. Another mutation known to result in resistance is a mutation in the *ethA* gene. The thioamides are more bactericidal than cycloserine or PAS. However, the bactericidal activity is only moderate and drug-related toxicity is often a limiting factor [11]. As with cycloserine, ramping up the dose over time is often done due to frequent side effects.

Para-Aminosalicylic Acid (PAS)

PAS is a bacteriostatic agent that is highly specific for *M. tuberculosis*. Resistance develops when mutations in *thyA* occur but these mutations account for only 6% of phenotypic resistance [42, 65]. Clinical trials demonstrated that monotherapy for 3 months produced clinical improvement with efficacy similar to that of streptomycin [66]. PAS in the form of an enteric-coated granule is widely used because of its improved gastric tolerance and lower dosage requirement [35]. However, this formulation requires refrigeration, which is not always available in developing countries. Fortunately, an enteric coated granule has been developed that does not require refrigeration. In contrast, PAS sodium lacks the requirement for refrigerated storage.

Drugs Not Ranked but Used

High-Dose Isoniazid

INH is a prodrug that must be activated by catalase-peroxidase in order to be active against *M. tuberculosis* [42]. Mutations within the *katG* gene result in moderate to high-level resistance to INH (MIC ≥ 16 μg/ml) and mutations in *inh*A and its promoter region cause lower levels of resistance (MIC 2–8 μg/ml) [67, 68]. Mutations in the *inh*A region also confer resistance to ethionamide and prothionamide [68–70]. Strains that are moderate to highly resistant to INH due to *katG* mutations are likely to be susceptible to ethionamide (prothionomide) whereas strains that demonstrate low-level resistance to INH (due to *inhA* mutations) are likely to be resistant to ethionamide and susceptible to high doses of INH [35, 71].

High-dose INH could potentially overcome low and moderate levels of resistance and provide an active drug for the treatment of MDR/RR-TB [72]. In a mouse model, an INH dose of 10 mg/kg/day exhibited bactericidal activity against an *inhA* promoter mutant and increasing the dose to 25 mg/kg/day resulted in bactericidal activity against a *katG* mutant [73]. In a randomized study evaluating the impact of high-dose INH (16–18 mg/kg) versus standard dose INH (5 mg/kg), patients who received high-dose INH became sputum smear-negative 2.4 times more rapidly and were 2.4 times more likely to achieve sputum culture conversion at 6 months than those who did not receive high-dose INH [74]. INH can produce drug-induced hepatotoxicity as well as peripheral neuropathy. However, in this study there was no increase in hepatotoxicity among patients that received high-dose INH but there was an increase in peripheral neuropathy (patients were not given supplementation with pyridoxine). Because of the low cost of INH and good reliability of drug susceptibility testing, high-dose INH should be considered as a possible drug in the treatment of patients with MDR/RR-TB who have INH resistance documented [35, 68, 72, 75]. High-dose INH is already being used in some treatment programs [76] and is a component of the shorter course MDR-TB regimen [32].

Pretomanid

Pretomanid (previously PA-824) is a novel oral bicyclic nitroimidazooxanine with bactericidal activity against *M. tuberculosis* with MICs ranging from 0.015 to 0.25 μg/ml, activity in a non-replicating model, and no cross-resistance with current antimicrobials (except for delamanid) used to treat MDR-TB [77]. Pretomanid kills actively replicating *M. tuberculosis* by inhibiting mycolic acid synthesis and kills non-replicating organisms by nitric oxide release [78, 79]. Pretomanid in combination with moxifloxacin and pyrazinamide or bedaquiline and pyrazinamide showed similar or better activity than a standard first-line regimen for drug-susceptible TB [80]. The combination of pretomanid-moxifloxacin-pyrazinamide had the best mean 14-day EBA (0.233), significantly higher than that of bedaquiline (0.061), bedaquiline-pyrazinamide (0.131), bedaquiline-PA-824 (0.114), but not PA-824-pyrazinamide (0.154), and comparable with that of standard treatment (0.140) [80]. A Phase 2b partly randomized study in patients with drug-susceptible TB or drug-resistant disease demonstrated that a combination of pretomanid, moxifloxacin, and pyrazinamide was bactericidal and well tolerated [81]. The combination of bedaquiline, pretomanid, and linezolid (BPaL regimen) was associated with good treatment success in a single-arm study of patients with XDR-TB or treatment intolerant or nonresponsive MDR-TB [26]. Based on this study, pretomanid was approved by the FDA in August 2019 for use with bedaquiline and linezold.

Use of Bedaquiline and Delamanid in Combination

Both bedaquiline and delamanid can prolong the QTc interval so there was initial reluctance to combine the two drugs. The endTB Observational study is a large multi-country cohort of patients with MDR/RR-TB who were treated with bedaquiline and/or delamanid [82]. A total of 1109 patients started bedaquiline (63%),

delamanid (27%), or both (10%). Overall, 85% experienced culture conversion within 6 months. Patients with cavitary disease and highly positive sputum smears had a lower probability of conversion relative to patients without either as did those with HIV infection.

A large global cohort including 52 centers from 29 countries and 883 patients reported that the proportion that culture converted was 92.8% in a median time of 60 days. Of 383 patients treated with bedaquiline (not delamanid) 74.2% achieved treatment success.

A systematic review reported 87 adult patients who were treated with both bedaquiline and delamanid, half of whom had XDR-TB [83]. Sputum culture conversion occurred in 81.4% of patients and the treatment success rate was 71.4%. Only 2.3% interrupted therapy due to life-threatening cardiac events.

A randomized phase 2 study enrolled 84 subjects with MDR/RR-TB in Peru and South Africa and assessed QTc intervals [84]. The mean change in QTc from baseline was 12.3 ms with bedaquiline, 8.6 ms with delamanid, and 20.7 ms with the combination. There were no Grade 3 or 4 adverse events and no deaths. By 24 weeks, culture conversion was 92% with bedaquiline, 91% with delamanid, and 95% with both.

3.3 Choosing Between a Longer or Shorter Regimen

The decision to choose a longer or shorter regimen is based on whether the isolate is susceptible to fluoroquinolones, previous treatment, availability of the drugs used in the regimen, severity and site of disease, and patient preference (Fig. 7.2). The WHO currently recommends three different all oral, shorter course regimens although in some circumstances a longer course regimen may be used. For example, patients with advanced forms of DR-TB (e.g., XDR-TB) or those who are not eligible or who

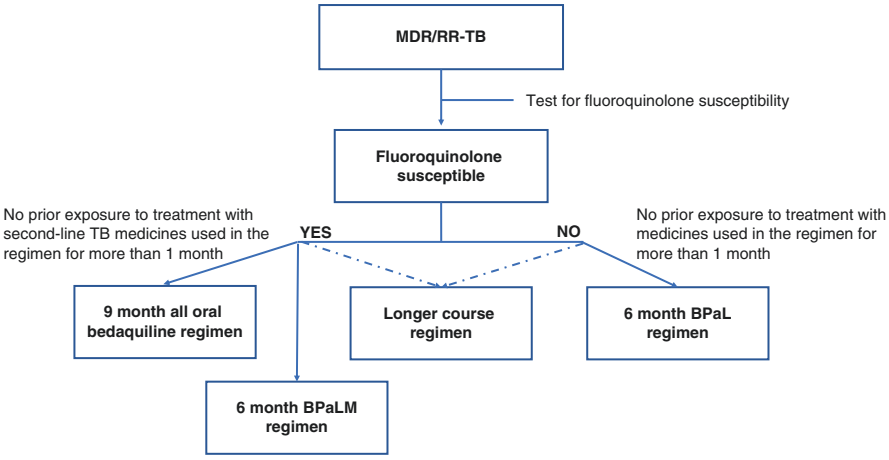

Fig. 7.2 Decision to use longer or shorter MDR/RR-TB regimens

have failed shorter treatment regimens will benefit from an individualized longer regimen designed using the WHO priority grouping of medicines (Table 7.2).

3.4 Designing a Longer Duration Treatment Regimen

Traditionally, MDR-TB treatment regimens are divided into an intensive phase and continuation phase. The intensive phase has been defined as the time that the injectable is being administered and the duration of the intensive phase can be defined by a fixed duration or time after culture conversion. The WHO recommends that the intensive phase is of 6–7 months duration with a total duration of 18–20 months duration or 15–17 months after culture conversion [7]. The ATS-led multi-society guidelines recommend an intensive phase of 5–7 months after culture conversion and a total duration of therapy of 15–21 months after culture conversion for MDR-TB and 15–24 months for pre-XDR or XDR-TB [6].

Current WHO guidelines recommend that at least four effective second-line drugs be included in the intensive phase of therapy and at least three drugs in the continuation phase if bedaquiline is stopped (Table 7.3) [7]. The regimen is designed

Table 7.3 Building a longer MDR/RR-TB regimen

ATS/CDC/IDSA/ERS approach		World Health Organization approach
Goal: ≥ 5 likely effective drugs in intensive phase and ≥4 in continuation phase	*Anti-TB drugs*	*Goal: ≥4 likely effective drugs and ≥3 after BDQ is stopped*
Step 1: Choose one later generation FQN	Levofloxacin or moxifloxacin	*Step 1*: Include all three medicines
Step 2: Choose both of these prioritized drugs	Bedaquiline Linezolid	
Step 3: Choose both of these prioritized drugs	Clofazimine Cycloserine/ terizidone	*Step 2*: Add one or both medicines
Step 4: Add to complete the regimen if necessary	Amikacin or streptomycin	*Step 3*: Add to complete the regimen and when medicines from Group A or B cannot be used
Step 5: If needed, or *oral agents preferred* over injectables	Ethambutol Delamanid Pyrazinamide	
Step 6: If limited options and cannot assemble a regimen of ≥5 effective drugs	Ethionamide or prothionamide Carbapenems with clavulanate P-aminosalicylic acid High-dose INH	

ATS American Thoracic Society, *CDC* Centers for Disease Control and Prevention, *ERS* European Respiratory Society, *IDSA* Infectious Diseases Society of America

Sources: Nahid P, et al. Treatment of drug-resistant tuberculosis. An official ATS/CDC/ERS/IDSA clinical practice guideline. Am J Respir Crit Care Med 2019;200:e93–e142. World Health Organization. Module 4. Treatment of drug-resistant tuberculosis

through a three-step process: (1) include all three medicine in Group A, (2) add one or both medicines from Group B, (3) add medicines from Group C to complete the regimen and when medicines from Groups A and B cannot be used. With an all oral regimen, there may not be a clear delineation between the intensive and continuation phase. For programs that administer bedaquiline for 6 months, then it is recommended that there be ≥3 drugs after bedaquiline is discontinued. If an injectable is used, the intensive phase should be of 6–7 months and the total duration of therapy should be 18–20 months or 15–17 months after culture conversion. The ATS-led multi-society guidelines recommends a six-step approach to designing a treatment regimen with the goal of including ≥5 drugs in the intensive phase and ≥4 drugs in the continuation phase [6].

3.5 Shorter Course Regimens

3.5.1 Standardized Shorter Course Injectable-Containing Regimen

The WHO first recommended a standardized shorter course regimen in their 2016 MDR-TB guideline although the regimen is no longer recommended [9]. This regimen was referred to as the "Bangladesh regimen" as the initial experience was reported in 2010 from Bangladesh [32]. In this study, patients with confirmed or suspected MDR-TB were assigned to one of six standardized treatment regimens studied in serial fashion. The last regimen to be studied, and the one with the best treatment outcomes, included 4 months of kanamycin, clofazimine, high-dose gatifloxacin (800 mg per day for those >50 kg), ethambutol, high-dose isoniazid (600 mg per day for those >50 kg), pyrazinamide, and prothionamide. The continuation phase was for 5 additional months (a total of 9 months) and included high-dose gatifloxacin, ethambutol, pyrazinamide, and clofazimine. This regimen was associated with treatment success of 87.8%, death in 5.3%, default in 5.8%, failure in 0.5%, and relapse in 0.5%.

The STREAM trial was a randomized controlled trial that enrolled patients from seven sites between July 2012 and June 2015 and followed them for 132 weeks post-randomization: patients were randomized to the shorter course regimen ($N = 282$) or longer conventional regimen ($N = 142$) [85]. A favorable outcome was marginally higher in the shorter regimen than the conventional regimen although not statistically significantly different (80.6 vs. 78.1%, $p = 0.6$). There were no statistically significant differences in other outcomes such as patient retention, time to culture conversion, relapse, or mortality. However, in the subgroup of patients with HIV infection, the number of observed deaths was higher in the shorter course arm compared to the control arm but again, the finding was not statistically significant [85].

3.5.2 Standardized Shorter Course Oral Bedaquiline-Containing Regimen

The current WHO guideline for the treatment of MDR/RR-TB recommends a shorter, all oral bedaquiline-containing regimen of 9–12 months duration for patients who have not been exposed to treatment with second-line TB medicines used in this regimen for more than a month, and in whom resistance to fluoroquinolones has been excluded [7]. In addition, the patient should not have extensive disease or severe extrapulmonary disease. The regimen can be used in people living with HIV. This regimen consists of 6 months of bedaquiline with 4–6 months of levofloxacin/moxifloxacin, clofazimine, pyrazinamide, ethambutol, high-dose isoniazid, and ethionamide followed by 5 months of levofloxacin/moxifloxacin, clofazimine, pyrazinamide, and ethambutol [7]. This is essentially the same regimen as was studied in the STREAM trial except bedaquiline is substituted for the aminoglycoside. Data to support the implementation of this shorter, all oral regimen comes from unpublished programmatic data in South Africa that was provided to the WHO. A total of 10,152 patients with MDR-TB between January and June, 2017 were analyzed: 891 patients received the shorter, all oral bedaquiline-containing regimen, 987 were treated with a shorter standardized regimen that included an injectable agent, 1437 were treated with longer regimens, and 474 were treated with longer regimens that included bedaquiline [7]. The use of the shorter, all oral bedaquiline-containing regimen was associated with higher treatment success rates and lower loss to follow-up than a shorter injectable-containing regimen or a longer bedaquiline-containing regimen. The regimen can be used in children aged 6 years and older, HIV infected individuals, and those with uncomplicated extrapulmonary disease. The all oral longer regimen was found to have a similar 6-month culture conversion as a longer injectable-containing regimen (83.8 vs. 85.5%) [86].

Since this recommendation was made, the regimen was modified by replacing ethionamide in the first two months with linezolid (600 mg). Based on a review of these data, the WHO now recommends the modified, linezolid-containing regimen [87].

3.5.3 BPaL and BPaLM Regimens

The WHO recommends that a new treatment regimen lasting 6 months and consisting of bedaquiline, pretomanid, and linezolid (BPaL) be used in MDR-TB patients whose isolate is resistant to fluoroquinolones, who have no previous exposure to bedaquiline and linezolid or have been exposed to the drugs for less than 1 month (Fig. 7.2) [87]. BPaL was studied in a single-arm, open-label (Nix-TB) trial in individuals with XDR-TB or treatment intolerant or nonresponsive MDR-TB [26]. The regimen was administered for 6 months with an option to extend it to 9 months for

slow bacteriologic responders. Among the 109 study participants, 62% had XDR-TB and 51% were HIV infected. Favorable outcomes were reported in 98 (90%) of the patients with unfavorable outcomes in 11 (10%). Of the latter, there were seven deaths, one withdrawal of consent, two relapses and one lost to follow-up. Unfortunately, adverse events were common with 81% of the cohort developing peripheral neuropathy and 48% cytopenias. Overall, only 18 (17.3%) of patients in the trial completed a full course of linezolid at the 1200 per day dose, 38 (36.5%) completed at 600 mg per day, 16 (15.4%) completed at 300 mg per day, 32 (30.7%) stopped linezolid due to an adverse event.

In order to evaluate alternative linezolid dosing strategies, the TB Alliance conducted the ZeNix trial which is a Phase 3, multicenter, and partially blinded randomized study [88]. Participants were randomized to one of four regimens: bedaquiline and pretomanid plus (1) linezolid 1200 mg daily for 26 weeks, (2) linezolid 600 mg daily for 26 weeks, (3) linezolid 1200 mg daily for 9 weeks, or (4) linezolid 600 mg for 9 weeks. All participants were treated for 6 months and the primary endpoint of the study was the incidence of bacteriologic failure, relapse, or clinical failure through follow-up until 6 months after the end of treatment. Favorable outcomes were reported in 93.2% of those taking linezolid 1200 mg for 26 weeks, 88.9% of those on linezolid 1200 mg for 9 weeks, 90.9% for those on linezolid 600 mg for 26 weeks and 84.1% for those on linezolid 600 mg for 9 weeks. Importantly, adverse events were less common with lower doses and shorter durations of therapy [88]. Based on these results it appears that a dose of 600 mg a day given for 26 weeks is highly effective with less adverse events than with 1200 mg daily.

BPaL was approved by the US FDA in August of 2019 and is being adopted in the USA with positive early experience [89]. The US Centers for Disease Control and Prevention recently published guidance for the use of BPaL noting that the regimen is approved for pulmonary disease and that pretomanid is not to be used outside of the BPaL regimen [90].

For patients whose isolate is susceptible to fluoroquinolones, WHO recommends a new PBaLM regimen. The data supporting this regimen comes from TB PRACTECAL which was a multi-arm, multi-stage, randomized, and controlled trial that evaluated the safety and efficacy of regimens containing bedaquiline, pretomanid, and linezolid (600 mg) for treatment of MDR/RR-TB. In Stage 1, participants were randomized to receive one of three experimental arms compared with the standard of care (longer WHO regimen). The best performing regimen (BPaLM) which was a regimen of bedaquiline, pretomanid, linezolid, and moxifloxacin administered for 6 months then moved on to Stage 2 in comparison with a standard longer WHO regimen. There were 242 patients enrolled in Belarus, South Africa, and Uzbekistan. The trial was stopped early due to an interim analysis demonstrating superiority of the experimental shorter regimen: 89% of the participants in the BPaLM arm were cured vs. 52% in the standard of care group (available at https://www.doctorswithoutborders.org).

4 Surgery

Both the WHO and the ATS-led guidelines recommend that elective partial lung resection (lobectomy or wedge resection) may be used with an appropriate treatment regimen in selected patients [6, 7]. Although there are no randomized studies assessing the added benefit of surgical resection over anti-TB chemotherapy alone, at least two systematic reviews and data from an individual patient meta-analysis have reported benefits in some patients. One systematic review reported the results of 15 observational studies [91] and another review reported the results of eight cohort studies of patients with MDR/XDR-TB and an additional 18 retrospective case series [92]. Treatment success varied between 45 and 77%; the median postoperative culture conversion was 93.5% (47–100%). Outcome data from 26 cohort studies (18 surgical studies and 8 nonsurgical studies) participating in the individual patient's meta-analysis used for development of the WHO recommendations showed a pooled treatment success of 84% with failure in 6%, relapse in 3%, death in 5% and default in 3% of patients [9]. In the analysis, a statistically significant improvement in cure and successful treatment was noted among patients who had surgery. However, this benefit was primarily seen in patients who had partial resection but not pneumonectomy.

Perioperative complications were reported in a median of 23% (0–39%) and perioperative mortality in 1.3% (0–5%). Risk factors that have been identified to increase the risk of postoperative bronchopleural fistula include positive cultures at the time of surgery, polymicrobial infections, right pneumonectomy, low FEV1, increased age, technique of bronchial closure, and endobronchial disease [91–93].

Based on these studies it appears that surgery for MDR/RR-TB can provide additional treatment benefits in selected patients but the procedure should only be performed by experienced surgeons after the patient has been on appropriate therapy for several months with the goal of achieving smear and/or culture conversion preoperatively, if possible. Prognosis appears to be better in those who underwent partial resection after culture conversion [9, 94].

5 Treatment Outcomes

Globally, treatment success is achieved in 59% of patients with MDR/RR-TB [2]. This outcome has slowly improved since 2012 when treatment success rates were reported by the WHO to be 50%. The slight improvement has been a result of small decreases in failure, mortality, and not evaluated. However, the proportion of those lost to follow-up has remained little changed. Hopefully, the adoption and implementation of shorter, all oral regimens will decrease those lost to follow-up and improve overall treatment success.

Several systematic reviews and meta-analyses have reported the pooled estimate of treatment success in patients with MDR-TB and XDR-TB [95–98]. A review

published by Bastos et al. [95] included 74 studies and 17,494 patients. Pooled treatment success was 60% in MDR-TB and 26% in XDR-TB. The number of drugs, specific drug, or duration of use was not associated with improved outcomes. MDR-TB patients receiving individualized treatments had better outcomes than those receiving standardized therapies (64 vs. 52%; $p < 0.0.01$). Note that the currently recommended all oral shorter course regimens have better treatment outcomes than longer injectable-containing regimens that made up the patients in the systematic reviews.

6 Special Situations

6.1 Extrapulmonary Disease

Current guidelines recommend that extrapulmonary TB be treated the same as pulmonary disease with the exception of TB meningitis. The ATS and Infectious Disease Society of America recommend extending the duration of therapy for patients with drug-susceptible CNS TB (12 months) or bone-joint disease (9 months). There are no specific recommendations for the treatment of MDR-TB involving an extrapulmonary site. However, if there is CNS involvement, it is critical to select drugs that penetrate the blood–brain barrier. For example, RIF, INH, PZA, prothionamide (ethionamide), cycloserine, linezolid, and later generation fluoroquinolones have good penetration into the CSF [99, 100]. The aminoglycosides and polypeptides penetrate into the CSF in the presence of inflammation so their benefit is likely to be greatest during the early phase of therapy. PAS and ethambutol have poor penetration into the CSF as do bedaquiline and clofazimine [99, 100]. There have been conflicting reports regarding delamanid [99, 101].

6.2 Children

Treatment of MDR-TB in children is often delayed due to difficulties in making a diagnosis because of the paucibacillary nature of the disease and difficulty obtaining adequate diagnostic specimens. These delays can result in disease progression, transmission to others, and death [76, 102]. Microbiologic confirmation of drug-resistant TB is difficult to obtain given the low bacterial load in children so treatment regimens are often designed based on the susceptibility pattern identified in the source case's isolate. Recommendations regarding treatment are based primarily on small observational studies and clinical/programmatic experience. Drug selection is similar to that in adults but the second-line drugs are not always produced in pediatric formulations or appropriate tablet size. In general, the treatment regimens are built the same way for children with MDR-TB as with adults although injectable

agents are used less frequently. Data supporting this approach comes from the fact that in children with clinically diagnosed TB (as opposed to bacteriologically confirmed) treatment success was high and not significantly different in patients treated with and without an injectable medication (93.5 vs. 98.1%) [9]. Data from an individual patient meta-analysis reported that 14% (119/842) of children treated for MDR-TB received injectable sparing regimens resulting in successful outcomes for 72% of those with confirmed MDR-TB and 94% of those with probable MD/RR-TB. Another systematic review and meta-analysis that included eight studies and 315 children with MDR/RR-TB reported the average duration of treatment ranged from 6 months to 34 months. The pooled estimate for treatment success (defined as a composite of cure and completion) was 81.7% with death in 5.9%, and default in 6.2% of patients [103]. Adverse reactions occurred in 39.1% of the children, the most common of which were nausea and vomiting followed by hearing loss, psychiatric effects, and hypothyroidism.

According to the WHO, children of any age can receive a 9–12 month all oral bedaquiline-containing regimen and children ≥6 years can receive a bedaquiline-containing longer regimen. Delamanid can be used in children of any age as part of a longer regimen [104, 105].

6.3 Pregnant Women

Treatment of MDR/RR-TB in pregnant women can be associated with adverse maternal and fetal outcomes. The ATS-led multi-society MDR-TB Guidelines performed a systematic review and identified 65 pregnant women for whom MDR-TB treatment outcome data were available of whom 32 (49%) were cured and 13 (20%) completed therapy for an overall treatment success of 69%. Fourteen percent of the women died. Fetal outcomes included 78.5% healthy births [6].

Another systematic review identified 12 studies including 174 pregnant women with MDR/RR-TB. The pooled prevalence was 7.5% for maternal death, 10.6% for pregnancy loss, 12.9% for preterm birth, and 23.7% for low birthweight [106]. Live birth outcome was significantly associated with the trimester of initiation of drug-resistant TB with the proportion of live births for the pregnancy trimester of 60, 90.0, and 100.0% for first, second, and third trimesters, respectively. The authors concluded that treatment for MDR/RR-TB should be delayed until after the first trimester [107].

Pregnant and lactating women were excluded from the NIX trial using the BPaL regimen so no data are available on the regimen in this setting. The WHO recommends using a longer regimen in pregnant and lactating women [7]. Breastfeeding is not recommended in women who are taking BPaL [7]. Infants born to women taking bedaquiline had lower mean birth weight compared with infants whose mothers did not take bedaquiline although this did not appear to be clinically significant [108].

Based on the available evidence, pregnant women with MDR/RR-TB should be treated including with second-line drugs. Most MDR-TB experts would avoid aminoglycosides and ethionamide and build a treatment regimen with alternative drugs.

6.4 People Living with HIV

Treatment of MDR/RR-TB in people with HIV infection is challenging because of the potential for overlapping toxicities, drug interactions, and development of IRIS. Inadequate treatment of HIV-infected patients with MDR/RR-TB has been associated with high mortality rates [109]. Thus, rapid identification of MDR/RR-TB with initiation of effective treatment regimens is critical. In addition, antiretroviral therapy is recommended for all HIV-infected patients with drug-resistant TB irrespective of CD4 cell count and treatment should be initiated as soon as possible, generally within the 2 weeks of initiating anti-TB therapy in those with CD4 counts <50 cells/mm^3 when TB meningitis is not suspected and within 8 weeks in those with higher CD4 counts. Data supporting this recommendation comes from several large, randomized trials [110–114]. Studies in drug-susceptible TB demonstrated that earlier initiation of ART (2 weeks after initiation of therapy) is associated with improved survival, particularly in those patients with CD4 cell counts less than 50 cells/mm^3 [110, 112]. The optimal time to initiate ART in someone with TB meningitis is uncertain. A study from Vietnam evaluating patients with advanced AIDS reported a higher incidence of potentially life-threatening adverse events among patients with TB meningitis starting early ART compared with those delaying ART until 2 months after initiating TB therapy [115]. Others suggest starting ART within the first 2 weeks in those with a CD4 count <50 cells/mm^3 and following the patient closely for signs of drug-related toxicities and CNS events [116].

Efavirenz should not be used with bedaquiline because of a significant reduction in bedaquiline serum concentration [116]. Lopinovir/ritonavir increases bedaquiline concentrations twofold but the clinical significance of this is not known. Dolutegravir has no known interactions with bedaquiline.

7 Monitoring for Treatment Response

The WHO recommends that treatment response be assessed by monthly sputum smear and culture rather than smear microscopy alone [7, 41] in order to identify failures earlier. Alternative modeling strategies were examined based on cohorts of MDR-TB from Estonia, Latvia, Philippines, Russia, and Peru from 2000 to 2004 [117]. Less than monthly monitoring results in delays in identifying conversion, which would prolong intensive therapy, hospitalization and respiratory isolation, increasing cost, and potential drug-related toxicity. Also, less frequent monitoring would delay detection of treatment failure.

8 Monitoring for Adverse Drug Reactions

Adverse events (AE) due to second ling drugs are very common during the course of treatment and they can negatively impact therapy resulting in default, morbidity, and even death (Table 7.1). Several systematic reviews have reported the frequency of adverse effects in those treated for MDR-TB [118–120] and several reviews have focused on specific drugs like clofazimine [29, 121, 122], cycloserine [123], linezolid [24, 124, 125], and carbapenems [126]. The individual patient meta-analysis that was used to group the second-line drugs based on an assessment of risks and benefits reported that 23.5% of patients had a least one drug permanently discontinued because of an adverse event and was associated with female sex, older age, and treatment in high-income countries [7]. In a study of over 5000 patients with MDR-TB, 57.3% reported at least one AE [120]. The three most common AEs were gastrointestinal disorders (32.1%), ototoxicity (14.6%), and psychiatric disorders (13.2%). For the 1519 patients for whom data were available regarding impact of AEs on MDR-TB therapy, 70.4% required a change in treatment. A systematic review (10 studies including 2776 of whom 70% were HIV infected) that focused on drug-resistant TB treatment in HIV prevalent areas reported that an average of 83% of patients experienced at least one AE and 24% experienced at least one serious AE [119]. There was no association between the occurrence of an AE and HIV infection.

Over 2000 patients received bedaquiline and delamanid in the endTB cohort and the most common AEs were peripheral neuropathy (26.4%), electrolyte depletion (26.0%), and hearing loss (13.2%) [127]. QTc interval prolongation occurred in 2.7%. Those who received linezolid or injectables were most likely to experience AEs. Hearing loss, acute renal failure, or electrolyte depletion occurred in 36.8% and peripheral neuropathy, optic neuritis, and/or myelosuppression occurred in 27.8% on linezolid.

9 Treatment of Contacts to MDR-TB

There have been no randomized controlled trials published addressing the safety and efficacy of treating contacts with MDR-TB although there are several observational cohorts that have been described in systematic reviews. The US Centers for Disease Control and Prevention published a systematic review and estimated MDR-TB incidence reduction was 90% based on five comparative studies using various preventive regimens including levofloxacin or moxifloxacin alone [128]. The ATS-led multi-society MDR-TB guideline performed a systematic review and meta-analysis and reported that 2 of 190 (1.1%) individuals treated for MDR-TB-related LTBI developed MDR-TB compared with 18 of 126 (14.3%) who received no treatment for LTBI resulting in a 90% reduction in incidence [6]. There was high (51%) discontinuation of pyrazinamide due to adverse effects and about one-third of those taking fluoroquinolones without pyrazinamide had adverse effects but only

l2% discontinued. Therefore, the guidelines suggest using a 6–12 month regimen with a fluoroquinolone alone or with a second drug based on susceptibility results. Due to the high rate of adverse events and discontinuations, pyrazinamide is not recommended as the second drug [6, 128]. A cost-effectiveness analysis reported savings in health systems and reduced mortality, the incidence of MDR-TB and incidence of acquired fluoroquinolone resistance as well as improved quality of life [129].

There are currently three randomized clinical trials enrolling adults and pediatric contacts of MDR-TB cases. Two of the trials are comparing levofloxacin with placebo and another trial is comparing delamanid with isoniazid.

10 Conclusion

So, what is the latest approach to treat drug-resistant pulmonary TB? This depends on a number of factors such as susceptibility to fluoroquinolones, previous treatment, availability of the drugs used in the regimen, severity and site of disease, and patient preference. For fluoroquinolone susceptible disease, a 6 month BPaLM regimen, 9 month all oral bedaquiline-containing regimen or traditional longer regimen are possible. For fluoroquinolone-resistant disease, BPaL or a longer regimen are recommended. With the current treatment options, injectable free, all oral regimens are preferred.

References

1. World Health Organization. Global tuberculosis report, 2020. Geneva: World Health Organization; 2020.
2. World Health Organization. Global tuberculosis report, 2021. Geneva: World Health Organization; 2021.
3. World Health Organization. Report of the meeting of the WHO Global Task Force on XDR-TB. Geneva: World Health Organization; 2006.
4. Roelens M, Battista Migliori G, Rozanova L, Estill J, Campbell JR, Cegielski JP, et al. Evidence-based definition for extensively drug-resistant tuberculosis. Am J Respir Crit Care Med. 2021;204(6):713–22. https://doi.org/10.1164/rccm.202009-3527OC.
5. Gegia M, Winters N, Benedetti A, van Soolingen D, Menzies D. Treatment of isoniazid-resistant tuberculosis with first-line drugs: a systematic review and meta-analysis. Lancet Infect Dis. 2017;17(2):223–34. https://doi.org/10.1016/S1473-3099(16)30407-8.
6. Nahid P, Mase SR, Migliori GB, Sotgiu G, Bothamley GH, Brozek JL, et al. Treatment of drug-resistant tuberculosis. An official ATS/CDC/ERS/IDSA clinical practice guideline. Am J Respir Crit Care Med. 2019;200(10):e93–e142. https://doi.org/10.1164/rccm.201909-1874ST.
7. World Health Organization. WHO consolidated guidelines on tuberculosis. Module 4: treatment—drug-resistant tuberculosis treatment. Geneva: World Health Organization; 2021.
8. Iseman MD. Treatment of multidrug-resistant tuberculosis. N Engl J Med. 1993;329(11):784–91. https://doi.org/10.1056/NEJM199309093291108.

9. World Health Organization. WHO treatment guidelines for drug-resistant tuberculosis, 2016 update. Geneva: World Health Organization; 2016.
10. Migliori GB, Lange C, Girardi E, Centis R, Besozzi G, Kliiman K, et al. Fluoroquinolones: are they essential to treat multidrug-resistant tuberculosis? Eur Respir J. 2008;31(4):904–5. https://doi.org/10.1183/09031936.00159807.
11. Caminero JA, Scardigli A. Classification of antituberculosis drugs: a new proposal based on the most recent evidence. Eur Respir J. 2015;46(4):887–93. https://doi.org/10.1183/13993003.00432-2015.
12. Chan ED, Laurel V, Strand MJ, Chan JF, Huynh ML, Goble M, et al. Treatment and outcome analysis of 205 patients with multidrug-resistant tuberculosis. Am J Respir Crit Care Med. 2004;169(10):1103–9. https://doi.org/10.1164/rccm.200308-1159OC.
13. Chiang CY, Enarson DA, Yu MC, Bai KJ, Huang RM, Hsu CJ, et al. Outcome of pulmonary multidrug-resistant tuberculosis: a 6-yr follow-up study. Eur Respir J. 2006;28(5):980–5. https://doi.org/10.1183/09031936.06.00125705.
14. Kam KM, Yip CW, Cheung TL, Tang HS, Leung OC, Chan MY. Stepwise decrease in moxifloxacin susceptibility amongst clinical isolates of multidrug-resistant Mycobacterium tuberculosis: correlation with ofloxacin susceptibility. Microb Drug Resist. 2006;12(1):7–11. https://doi.org/10.1089/mdr.2006.12.7.
15. Cheng AF, Yew WW, Chan EW, Chin ML, Hui MM, Chan RC. Multiplex PCR amplimer conformation analysis for rapid detection of gyrA mutations in fluoroquinolone-resistant Mycobacterium tuberculosis clinical isolates. Antimicrob Agents Chemother. 2004;48(2):596–601.
16. Zignol M, Dean AS, Alikhanova N, Andres S, Cabibbe AM, Cirillo DM, et al. Population-based resistance of *Mycobacterium tuberculosis* isolates to pyrazinamide and fluoroquinolones: results from a multicountry surveillance project. Lancet Infect Dis. 2016;16(10):1185–92. https://doi.org/10.1016/S1473-3099(16)30190-6.
17. Johnson JL, Hadad DJ, Boom WH, Daley CL, Peloquin CA, Eisenach KD, et al. Early and extended early bactericidal activity of levofloxacin, gatifloxacin and moxifloxacin in pulmonary tuberculosis. Int J Tuberc Lung Dis. 2006;10(6):605–12.
18. Peloquin CA, Hadad DJ, Molino LP, Palaci M, Boom WH, Dietze R, et al. Population pharmacokinetics of levofloxacin, gatifloxacin, and moxifloxacin in adults with pulmonary tuberculosis. Antimicrob Agents Chemother. 2008;52(3):852–7. https://doi.org/10.1128/AAC.01036-07.
19. Lee J, Lee CH, Kim DK, Yoon HI, Kim JY, Lee SM, et al. Retrospective comparison of levofloxacin and moxifloxacin on multidrug-resistant tuberculosis treatment outcomes. Korean J Intern Med. 2011;26(2):153–9. https://doi.org/10.3904/kjim.2011.26.2.153.
20. Diacon AH, Pym A, Grobusch M, Patientia R, Rustomjee R, Page-Shipp L, et al. The diarylquinoline TMC207 for multidrug-resistant tuberculosis. N Engl J Med. 2009;360(23):2397–405. https://doi.org/10.1056/NEJMoa0808427.
21. Diacon AH, Donald PR, Pym A, Grobusch M, Patientia RF, Mahanyele R, et al. Randomized pilot trial of eight weeks of bedaquiline (TMC207) treatment for multidrug-resistant tuberculosis: long-term outcome, tolerability, and effect on emergence of drug resistance. Antimicrob Agents Chemother. 2012;56(6):3271–6. https://doi.org/10.1128/AAC.06126-11.
22. Pym AS, Diacon AH, Tang SJ, Conradie F, Danilovits M, Chuchottaworn C, et al. Bedaquiline in the treatment of multidrug- and extensively drug-resistant tuberculosis. Eur Respir J. 2016;47(2):564–74. https://doi.org/10.1183/13993003.00724-2015.
23. Borisov SE, Dheda K, Enwerem M, Romero Leyet R, D'Ambrosio L, Centis R, et al. Effectiveness and safety of bedaquiline-containing regimens in the treatment of MDR- and XDR-TB: a multicentre study. Eur Respir J. 2017;49(5):1700387. https://doi.org/10.1183/13993003.00387-2017.
24. Sotgiu G, Centis R, D'Ambrosio L, Alffenaar JW, Anger HA, Caminero JA, et al. Efficacy, safety and tolerability of linezolid containing regimens in treating MDR-TB and XDR-TB:

systematic review and meta-analysis. Eur Respir J. 2012;40(6):1430–42. https://doi.org/10.1183/09031936.00022912.
25. Cox H, Ford N. Linezolid for the treatment of complicated drug-resistant tuberculosis: a systematic review and meta-analysis. Int J Tuberc Lung Dis. 2012;16(4):447–54. https://doi.org/10.5588/ijtld.11.0451.
26. Conradie F, Diacon AH, Ngubane N, Howell P, Everitt D, Crook AM, et al. Treatment of highly drug-resistant pulmonary tuberculosis. N Engl J Med. 2020;382(10):893–902. https://doi.org/10.1056/NEJMoa1901814.
27. Dooley KE, Obuku EA, Durakovic N, Belitsky V, Mitnick C, Nuermberger EL, et al. World Health Organization group 5 drugs for the treatment of drug-resistant tuberculosis: unclear efficacy or untapped potential? J Infect Dis. 2013;207(9):1352–8. https://doi.org/10.1093/infdis/jis460.
28. Xu J, Lu Y, Fu L, Zhu H, Wang B, Mdluli K, et al. In vitro and in vivo activity of clofazimine against *Mycobacterium tuberculosis* persisters. Int J Tuberc Lung Dis. 2012;16(8):1119–25. https://doi.org/10.5588/ijtld.11.0752.
29. Dey T, Brigden G, Cox H, Shubber Z, Cooke G, Ford N. Outcomes of clofazimine for the treatment of drug-resistant tuberculosis: a systematic review and meta-analysis. J Antimicrob Chemother. 2013;68(2):284–93. https://doi.org/10.1093/jac/dks389.
30. Xu HB, Jiang RH, Xiao HP. Clofazimine in the treatment of multidrug-resistant tuberculosis. Clin Microbiol Infect. 2012;18(11):1104–10. https://doi.org/10.1111/j.1469-0691.2011.03716.x.
31. Dalcolmo M, Gayoso R, Sotgiu G, D'Ambrosio L, Rocha JL, Borga L, et al. Effectiveness and safety of clofazimine in multidrug-resistant tuberculosis: a nationwide report from Brazil. Eur Respir J. 2017;49(3):1602445. https://doi.org/10.1183/13993003.02445-2016.
32. Van Deun A, Maug AK, Salim MA, Das PK, Sarker MR, Daru P, et al. Short, highly effective, and inexpensive standardized treatment of multidrug-resistant tuberculosis. Am J Respir Crit Care Med. 2010;182(5):684–92. https://doi.org/10.1164/rccm.201001-0077OC.
33. Van Deun A, Salim MA, Das AP, Bastian I, Portaels F. Results of a standardised regimen for multidrug-resistant tuberculosis in Bangladesh. Int J Tuberc Lung Dis. 2004;8(5):560–7.
34. Tang S, Yao L, Hao X, Liu Y, Zeng L, Liu G, et al. Clofazimine for the treatment of multidrug-resistant tuberculosis: prospective, multicenter, randomized controlled study in China. Clin Infect Dis. 2015;60(9):1361–7. https://doi.org/10.1093/cid/civ027.
35. Caminero JA, Sotgiu G, Zumla A, Migliori GB. Best drug treatment for multidrug-resistant and extensively drug-resistant tuberculosis. Lancet Infect Dis. 2010;10(9):621–9. https://doi.org/10.1016/S1473-3099(10)70139-0.
36. Epstein IG, Nair KG, Boyd LJ, Auspitz P. Cycloserine-isoniazid combination therapy in virgin cases of pulmonary tuberculosis. Dis Chest. 1958;33(4):371–81.
37. Angel JH, Bhatia AL, Devadatta S, Fox W, Janardhanam B, Radhakrishna S, et al. A controlled comparison of cycloserine plus ethionamide with cycloserine plus thiacetazone in patients with active pulmonary tuberculosis despite prolonged previous chemotherapy. Tubercle. 1963;44:215–24.
38. Shi W, Zhang X, Jiang X, Yuan H, Lee JS, Barry CE 3rd, et al. Pyrazinamide inhibits trans-translation in *Mycobacterium tuberculosis*. Science. 2011;333(6049):1630–2. https://doi.org/10.1126/science.1208813.
39. Mitnick C, Bayona J, Palacios E, Shin S, Furin J, Alcantara F, et al. Community-based therapy for multidrug-resistant tuberculosis in Lima, Peru. N Engl J Med. 2003;348(2):119–28. https://doi.org/10.1056/NEJMoa022928.
40. Migliori GB, Besozzi G, Girardi E, Kliiman K, Lange C, Toungoussova OS, et al. Clinical and operational value of the extensively drug-resistant tuberculosis definition. Eur Respir J. 2007;30(4):623–6. https://doi.org/10.1183/09031936.00077307.
41. World Health Organization. Guidelines for the programmatic management of drug-reisistant tuberculosis. Geneva: World Health Organization; 2011.

42. Dooley KE, Mitnick CD, Ann DeGroote M, Obuku E, Belitsky V, Hamilton CD, et al. Old drugs, new purpose: retooling existing drugs for optimized treatment of resistant tuberculosis. Clin Infect Dis. 2012;55(4):572–81. https://doi.org/10.1093/cid/cis487.
43. World Health Organization. Guidelines for the programmatic management of drug-resistant tuberculosis. Geneva: World Health Organization; 2008.
44. Gler MT, Skripconoka V, Sanchez-Garavito E, Xiao H, Cabrera-Rivero JL, Vargas-Vasquez DE, et al. Delamanid for multidrug-resistant pulmonary tuberculosis. N Engl J Med. 2012;366(23):2151–60. https://doi.org/10.1056/NEJMoa1112433.
45. von Groote-Bidlingmaier F, Patientia R, Sanchez E, Balanag V Jr, Ticona E, Segura P, et al. Efficacy and safety of delamanid in combination with an optimised background regimen for treatment of multidrug-resistant tuberculosis: a multicentre, randomised, double-blind, placebo-controlled, parallel group phase 3 trial. Lancet Respir Med. 2019;7(3):249–59. https://doi.org/10.1016/S2213-2600(18)30426-0.
46. Chang KC, Chung-Ching Leung E, Law WS, Leung WM, Tai LB, Lee SN, et al. Early experience with delamanid-containing regimens in the treatment of complicated multidrug-resistant tuberculosis in Hong Kong. Eur Respir J. 2018;51(6):1800159. https://doi.org/10.1183/13993003.00159-2018.
47. Kuksa L, Barkane L, Hittel N, Gupta R. Final treatment outcomes of multidrug- and extensively drug-resistant tuberculosis patients in Latvia receiving delamanid-containing regimens. Eur Respir J. 2017;50(5):1701105. https://doi.org/10.1183/13993003.01105-2017.
48. Hewison C, Ferlazzo G, Avaliani Z, Hayrapetyan A, Jonckheere S, Khaidarkhanova Z, et al. Six-month response to delamanid treatment in MDR TB patients. Emerg Infect Dis. 2017;23(10):1746–8. https://doi.org/10.3201/eid2310.170468.
49. Hafkin J, Hittel N, Martin A, Gupta R. Early outcomes in MDR-TB and XDR-TB patients treated with delamanid under compassionate use. Eur Respir J. 2017;50(1):1700311. https://doi.org/10.1183/13993003.00311-2017.
50. Chambers HF, Turner J, Schecter GF, Kawamura M, Hopewell PC. Imipenem for treatment of tuberculosis in mice and humans. Antimicrob Agents Chemother. 2005;49(7):2816–21. https://doi.org/10.1128/AAC.49.7.2816-2821.2005.
51. De Lorenzo S, Alffenaar JW, Sotgiu G, Centis R, D'Ambrosio L, Tiberi S, et al. Efficacy and safety of meropenem-clavulanate added to linezolid-containing regimens in the treatment of MDR-/XDR-TB. Eur Respir J. 2013;41(6):1386–92. https://doi.org/10.1183/09031936.00124312.
52. Tiberi S, Payen MC, Sotgiu G, D'Ambrosio L, Alarcon Guizado V, Alffenaar JW, et al. Effectiveness and safety of meropenem/clavulanate-containing regimens in the treatment of MDR- and XDR-TB. Eur Respir J. 2016;47(4):1235–43. https://doi.org/10.1183/13993003.02146-2015.
53. Tiberi S, Sotgiu G, D'Ambrosio L, Centis R, Abdo Arbex M, Alarcon Arrascue E, et al. Comparison of effectiveness and safety of imipenem/clavulanate- versus meropenem/clavulanate-containing regimens in the treatment of MDR- and XDR-TB. Eur Respir J. 2016;47(6):1758–66. https://doi.org/10.1183/13993003.00214-2016.
54. Tiberi S, Sotgiu G, D'Ambrosio L, Centis R, Arbex MA, Alarcon Arrascue E, et al. Effectiveness and safety of imipenem-clavulanate added to an optimized background regimen (OBR) versus OBR control regimens in the treatment of multidrug-resistant and extensively drug-resistant tuberculosis. Clin Infect Dis. 2016;62(9):1188–90. https://doi.org/10.1093/cid/ciw088.
55. Tiberi S, D'Ambrosio L, De Lorenzo S, Viggiani P, Centis R, Sotgiu G, et al. Ertapenem in the treatment of multidrug-resistant tuberculosis: first clinical experience. Eur Respir J. 2016;47(1):333–6. https://doi.org/10.1183/13993003.01278-2015.
56. Crowle AJ, Sbarbaro JA, Judson FN, Douvas GS, May MH. Inhibition by streptomycin of tubercle bacilli within cultured human macrophages. Am Rev Respir Dis. 1984;130(5):839–44. https://doi.org/10.1164/arrd.1984.130.5.839.

57. Caminero JA, World Health O, American Thoracic S, British Thoracic S. Treatment of multidrug-resistant tuberculosis: evidence and controversies. Int J Tuberc Lung Dis. 2006;10(8):829–37.
58. Heifets L, Lindholm-Levy P. Comparison of bactericidal activities of streptomycin, amikacin, kanamycin, and capreomycin against *Mycobacterium avium* and *M. tuberculosis*. Antimicrob Agents Chemother. 1989;33(8):1298–301.
59. Seddon JA, Godfrey-Faussett P, Jacobs K, Ebrahim A, Hesseling AC, Schaaf HS. Hearing loss in patients on treatment for drug-resistant tuberculosis. Eur Respir J. 2012;40(5):1277–86. https://doi.org/10.1183/09031936.00044812.
60. Reuter A, Tisile P, von Delft D, Cox H, Cox V, Ditiu L, et al. The devil we know: is the use of injectable agents for the treatment of MDR-TB justified? Int J Tuberc Lung Dis. 2017;21(11):1114–26. https://doi.org/10.5588/ijtld.17.0468.
61. Georghiou SB, Magana M, Garfein RS, Catanzaro DG, Catanzaro A, Rodwell TC. Evaluation of genetic mutations associated with *Mycobacterium tuberculosis* resistance to amikacin, kanamycin and capreomycin: a systematic review. PLoS One. 2012;7(3):e33275. https://doi.org/10.1371/journal.pone.0033275.
62. Alangaden GJ, Kreiswirth BN, Aouad A, Khetarpal M, Igno FR, Moghazeh SL, et al. Mechanism of resistance to amikacin and kanamycin in *Mycobacterium tuberculosis*. Antimicrob Agents Chemother. 1998;42(5):1295–7.
63. Kruuner A, Jureen P, Levina K, Ghebremichael S, Hoffner S. Discordant resistance to kanamycin and amikacin in drug-resistant *Mycobacterium tuberculosis*. Antimicrob Agents Chemother. 2003;47(9):2971–3.
64. Maus CE, Plikaytis BB, Shinnick TM. Molecular analysis of cross-resistance to capreomycin, kanamycin, amikacin, and viomycin in *Mycobacterium tuberculosis*. Antimicrob Agents Chemother. 2005;49(8):3192–7. https://doi.org/10.1128/AAC.49.8.3192-3197.2005.
65. Mathys V, Wintjens R, Lefevre P, Bertout J, Singhal A, Kiass M, et al. Molecular genetics of para-aminosalicylic acid resistance in clinical isolates and spontaneous mutants of *Mycobacterium tuberculosis*. Antimicrob Agents Chemother. 2009;53(5):2100–9. https://doi.org/10.1128/AAC.01197-08.
66. PARA-AMINOSALICYLIC acid treatment in pulmonary tuberculosis. Am Rev Tuberc. 1950;61(5):597–612.
67. Gagneux S, Burgos MV, DeRiemer K, Encisco A, Munoz S, Hopewell PC, et al. Impact of bacterial genetics on the transmission of isoniazid-resistant *Mycobacterium tuberculosis*. PLoS Pathog. 2006;2(6):e61. https://doi.org/10.1371/journal.ppat.0020061.
68. Cambau E, Viveiros M, Machado D, Raskine L, Ritter C, Tortoli E, et al. Revisiting susceptibility testing in MDR-TB by a standardized quantitative phenotypic assessment in a European multicentre study. J Antimicrob Chemother. 2015;70(3):686–96. https://doi.org/10.1093/jac/dku438.
69. Morlock GP, Metchock B, Sikes D, Crawford JT, Cooksey RC. ethA, inhA, and katG loci of ethionamide-resistant clinical *Mycobacterium tuberculosis* isolates. Antimicrob Agents Chemother. 2003;47(12):3799–805.
70. Banerjee A, Dubnau E, Quemard A, Balasubramanian V, Um KS, Wilson T, et al. inhA, a gene encoding a target for isoniazid and ethionamide in Mycobacterium tuberculosis. Science. 1994;263(5144):227–30.
71. Warren RM, Streicher EM, Gey van Pittius NC, Marais BJ, van der Spuy GD, Victor TC, et al. The clinical relevance of Mycobacterial pharmacogenetics. Tuberculosis (Edinb). 2009;89(3):199–202. https://doi.org/10.1016/j.tube.2009.03.001.
72. Rieder HL, Van Deun A. Rationale for high-dose isoniazid in the treatment of multidrug-resistant tuberculosis. Int J Tuberc Lung Dis. 2017;21(1):123–4. https://doi.org/10.5588/ijtld.16.0619.
73. Cynamon MH, Zhang Y, Harpster T, Cheng S, DeStefano MS. High-dose isoniazid therapy for isoniazid-resistant murine *Mycobacterium tuberculosis* infection. Antimicrob Agents Chemother. 1999;43(12):2922–4.

74. Katiyar SK, Bihari S, Prakash S, Mamtani M, Kulkarni H. A randomised controlled trial of high-dose isoniazid adjuvant therapy for multidrug-resistant tuberculosis. Int J Tuberc Lung Dis. 2008;12(2):139–45.
75. Caminero JA, Piubello A, Scardigli A, Migliori GB. Proposal for a standardised treatment regimen to manage pre- and extensively drug-resistant tuberculosis cases. Eur Respir J. 2017;50(1):1700648. https://doi.org/10.1183/13993003.00648-2017.
76. Schaaf HS, Moll AP, Dheda K. Multidrug- and extensively drug-resistant tuberculosis in Africa and South America: epidemiology, diagnosis and management in adults and children. Clin Chest Med. 2009;30(4):667–83, vii-viii. https://doi.org/10.1016/j.ccm.2009.08.019.
77. Stover CK, Warrener P, VanDevanter DR, Sherman DR, Arain TM, Langhorne MH, et al. A small-molecule nitroimidazopyran drug candidate for the treatment of tuberculosis. Nature. 2000;405(6789):962–6. https://doi.org/10.1038/35016103.
78. Singh R, Manjunatha U, Boshoff HI, Ha YH, Niyomrattanakit P, Ledwidge R, et al. PA-824 kills nonreplicating *Mycobacterium tuberculosis* by intracellular NO release. Science. 2008;322(5906):1392–5. https://doi.org/10.1126/science.1164571.
79. Manjunatha U, Boshoff HI, Barry CE. The mechanism of action of PA-824: novel insights from transcriptional profiling. Commun Integr Biol. 2009;2(3):215–8. https://doi.org/10.4161/cib.2.3.7926.
80. Diacon AH, Dawson R, von Groote-Bidlingmaier F, Symons G, Venter A, Donald PR, et al. 14-day bactericidal activity of PA-824, bedaquiline, pyrazinamide, and moxifloxacin combinations: a randomised trial. Lancet. 2012;380(9846):986–93. https://doi.org/10.1016/S0140-6736(12)61080-0.
81. Dawson R, Diacon AH, Everitt D, van Niekerk C, Donald PR, Burger DA, et al. Efficiency and safety of the combination of moxifloxacin, pretomanid (PA-824), and pyrazinamide during the first 8 weeks of antituberculosis treatment: a phase 2b, open-label, partly randomised trial in patients with drug-susceptible or drug-resistant pulmonary tuberculosis. Lancet. 2015;385(9979):1738–47. https://doi.org/10.1016/S0140-6736(14)62002-X.
82. Franke MF, Khan P, Hewison C, Khan U, Huerga H, Seung KJ, et al. Culture conversion in patients treated with bedaquiline and/or delamanid. A prospective multicountry study. Am J Respir Crit Care Med. 2021;203(1):111–9. https://doi.org/10.1164/rccm.202001-0135OC.
83. Pontali E, Sotgiu G, Tiberi S, Tadolini M, Visca D, D'Ambrosio L, et al. Combined treatment of drug-resistant tuberculosis with bedaquiline and delamanid: a systematic review. Eur Respir J. 2018;52(1) https://doi.org/10.1183/13993003.00934-2018.
84. Dooley KE, Rosenkranz SL, Conradie F, Moran L, Hafner R, von Groote-Bidlingmaier F, et al. QT effects of bedaquiline, delamanid, or both in patients with rifampicin-resistant tuberculosis: a phase 2, open-label, randomised, controlled trial. Lancet Infect Dis. 2021;21(7):975–83. https://doi.org/10.1016/S1473-3099(20)30770-2.
85. Nunn AJ, Phillips PPJ, Meredith SK, Chiang CY, Conradie F, Dalai D, et al. A trial of a shorter regimen for rifampin-resistant tuberculosis. N Engl J Med. 2019;380(13):1201–13. https://doi.org/10.1056/NEJMoa1811867.
86. Khan PY, Franke MF, Hewison C, Seung KJ, Huerga H, Atwood S, et al. All-oral longer regimens are effective for the management of multidrug-resistant tuberculosis in high-burden settings. Eur Respir J. 2022;59(1):2004345. https://doi.org/10.1183/13993003.04345-2020.
87. World Health Organization. Rapid communication: key changes to the treatment of drug-resistant tuberculosis. Geneva: World Health Organization; 2022.
88. Conradie F ED, Olugbosi M, Wills G, Fabiane S, Timm J, Spigelman M. High rate of successful outcomes treating highly resistant TB in the ZeNix study of pretomanid, bedaquiline, and alternative doses and duration of linezolid. Conference abstract: International AIDS Society. 2021.
89. Haley CA, Macias P, Jasuja S, Jones BA, Rowlinson MC, Jaimon R, et al. Novel 6-month treatment for drug-resistant tuberculosis, United States. Emerg Infect Dis. 2021;27(1):332–4. https://doi.org/10.3201/eid2701.203766.

90. Centers for Disease Control and Prevention. Provisional CDC guidance for the use of pretomanid as part of a regimen [bedaquiline, pretomanid, and linezolid (BPaL)] to treat drug-resistant tuberculosis disease. Atlanta: Centers for Disease Control and Prevention; 2022.
91. Xu HB, Jiang RH, Li L. Pulmonary resection for patients with multidrug-resistant tuberculosis: systematic review and meta-analysis. J Antimicrob Chemother. 2011;66(8):1687–95. https://doi.org/10.1093/jac/dkr210.
92. Kempker RR, Vashakidze S, Solomonia N, Dzidzikashvili N, Blumberg HM. Surgical treatment of drug-resistant tuberculosis. Lancet Infect Dis. 2012;12(2):157–66. https://doi.org/10.1016/S1473-3099(11)70244-4.
93. Pomerantz BJ, Cleveland JC Jr, Olson HK, Pomerantz M. Pulmonary resection for multidrug resistant tuberculosis. J Thorac Cardiovasc Surg. 2001;121(3):448–53. https://doi.org/10.1067/mtc.2001.112339.
94. Fox GJ, Mitnick CD, Benedetti A, Chan ED, Becerra M, Chiang CY, et al. Surgery as an adjunctive treatment for multidrug-resistant tuberculosis: an individual patient data meta-analysis. Clin Infect Dis. 2016;62(7):887–95. https://doi.org/10.1093/cid/ciw002.
95. Bastos ML, Lan Z, Menzies D. An updated systematic review and meta-analysis for treatment of multidrug-resistant tuberculosis. Eur Respir J. 2017;49(3):1600803. https://doi.org/10.1183/13993003.00803-2016.
96. Johnston JC, Shahidi NC, Sadatsafavi M, Fitzgerald JM. Treatment outcomes of multidrug-resistant tuberculosis: a systematic review and meta-analysis. PLoS One. 2009;4(9):e6914. https://doi.org/10.1371/journal.pone.0006914.
97. Orenstein EW, Basu S, Shah NS, Andrews JR, Friedland GH, Moll AP, et al. Treatment outcomes among patients with multidrug-resistant tuberculosis: systematic review and meta-analysis. Lancet Infect Dis. 2009;9(3):153–61. https://doi.org/10.1016/S1473-3099(09)70041-6.
98. Jacobson KR, Tierney DB, Jeon CY, Mitnick CD, Murray MB. Treatment outcomes among patients with extensively drug-resistant tuberculosis: systematic review and meta-analysis. Clin Infect Dis. 2010;51(1):6–14. https://doi.org/10.1086/653115.
99. Kempker RR, Smith AGC, Avaliani T, Gujabidze M, Bakuradze T, Sabanadze S, et al. Cycloserine and linezolid for tuberculosis meningitis: pharmacokinetic evidence of potential usefulness. Clin Infect Dis. 2021; https://doi.org/10.1093/cid/ciab992.
100. Donald PR. Cerebrospinal fluid concentrations of antituberculosis agents in adults and children. Tuberculosis (Edinb). 2010;90(5):279–92. https://doi.org/10.1016/j.tube.2010.07.002.
101. Tucker EW, Pieterse L, Zimmerman MD, Udwadia ZF, Peloquin CA, Gler MT, et al. Delamanid central nervous system pharmacokinetics in tuberculous meningitis in rabbits and humans. Antimicrob Agents Chemother. 2019;63(10):e00913–9. https://doi.org/10.1128/AAC.00913-19.
102. Seddon JA, Hesseling AC, Marais BJ, McIlleron H, Peloquin CA, Donald PR, et al. Paediatric use of second-line anti-tuberculosis agents: a review. Tuberculosis (Edinb). 2012;92(1):9–17. https://doi.org/10.1016/j.tube.2011.11.001.
103. Ettehad D, Schaaf HS, Seddon JA, Cooke GS, Ford N. Treatment outcomes for children with multidrug-resistant tuberculosis: a systematic review and meta-analysis. Lancet Infect Dis. 2012;12(6):449–56. https://doi.org/10.1016/S1473-3099(12)70033-6.
104. World Health Organization. Rapid communication on updated guidance on the management of tuberculosis in children and adolescents. Geneva: World Health Organization; 2021.
105. World Health Organization. WHO consolidated guidelines on tuberculosis. Module 5: management of tuberculosis in children and adolescents. Geneva: World Health Organization; 2022.
106. Alene KA, Jegnie A, Adane AA. Multidrug-resistant tuberculosis during pregnancy and adverse birth outcomes: a systematic review and meta-analysis. BJOG. 2021;128(7):1125–33. https://doi.org/10.1111/1471-0528.16573.
107. Walt MV, Masuku S, Botha S, Nkwenika T, Keddy KH. Retrospective record review of pregnant women treated for rifampicin-resistant tuberculosis in South Africa. PLoS One. 2020;15(9):e0239018. https://doi.org/10.1371/journal.pone.0239018.

108. Loveday M, Hughes J, Sunkari B, Master I, Hlangu S, Reddy T, et al. Maternal and infant outcomes among pregnant women treated for multidrug/rifampicin-resistant tuberculosis in South Africa. Clin Infect Dis. 2021;72(7):1158–68. https://doi.org/10.1093/cid/ciaa189.
109. Gandhi NR, Moll A, Sturm AW, Pawinski R, Govender T, Lalloo U, et al. Extensively drug-resistant tuberculosis as a cause of death in patients co-infected with tuberculosis and HIV in a rural area of South Africa. Lancet. 2006;368(9547):1575–80. https://doi.org/10.1016/S0140-6736(06)69573-1.
110. Abdool Karim SS, Naidoo K, Grobler A, Padayatchi N, Baxter C, Gray AL, et al. Integration of antiretroviral therapy with tuberculosis treatment. N Engl J Med. 2011;365(16):1492–501. https://doi.org/10.1056/NEJMoa1014181.
111. Havlir DV, Kendall MA, Ive P, Kumwenda J, Swindells S, Qasba SS, et al. Timing of antiretroviral therapy for HIV-1 infection and tuberculosis. N Engl J Med. 2011;365(16):1482–91. https://doi.org/10.1056/NEJMoa1013607.
112. Blanc FX, Sok T, Laureillard D, Borand L, Rekacewicz C, Nerrienet E, et al. Earlier versus later start of antiretroviral therapy in HIV-infected adults with tuberculosis. N Engl J Med. 2011;365(16):1471–81. https://doi.org/10.1056/NEJMoa1013911.
113. Mfinanga SG, Kirenga BJ, Chanda DM, Mutayoba B, Mthiyane T, Yimer G, et al. Early versus delayed initiation of highly active antiretroviral therapy for HIV-positive adults with newly diagnosed pulmonary tuberculosis (TB-HAART): a prospective, international, randomised, placebo-controlled trial. Lancet Infect Dis. 2014;14(7):563–71. https://doi.org/10.1016/S1473-3099(14)70733-9.
114. Luetkemeyer AF, Kendall MA, Nyirenda M, Wu X, Ive P, Benson CA, et al. Tuberculosis immune reconstitution inflammatory syndrome in A5221 STRIDE: timing, severity, and implications for HIV-TB programs. J Acquir Immune Defic Syndr. 2014;65(4):423–8. https://doi.org/10.1097/QAI.0000000000000030.
115. Torok ME, Yen NT, Chau TT, Mai NT, Phu NH, Mai PP, et al. Timing of initiation of antiretroviral therapy in human immunodeficiency virus (HIV)—associated tuberculous meningitis. Clin Infect Dis. 2011;52(11):1374–83. https://doi.org/10.1093/cid/cir230.
116. 2022.https://clinicalinfo.hiv.gov/en/guideline/adult-andd-adolescent-opportunistic-infection/
117. Kurbatova EV, Gammino VM, Bayona J, Becerra M, Danilovitz M, Falzon D, et al. Frequency and type of microbiological monitoring of multidrug-resistant tuberculosis treatment. Int J Tuberc Lung Dis. 2011;15(11):1553–5, i. https://doi.org/10.5588/ijtld.11.0101.
118. Lan Z, Ahmad N, Baghaei P, Barkane L, Benedetti A, Brode SK, et al. Drug-associated adverse events in the treatment of multidrug-resistant tuberculosis: an individual patient data meta-analysis. Lancet Respir Med. 2020;8(4):383–94. https://doi.org/10.1016/S2213-2600(20)30047-3.
119. Schnippel K, Firnhaber C, Berhanu R, Page-Shipp L, Sinanovic E. Adverse drug reactions during drug-resistant TB treatment in high HIV prevalence settings: a systematic review and meta-analysis. J Antimicrob Chemother. 2017;72(7):1871–9. https://doi.org/10.1093/jac/dkx107.
120. Wu S, Zhang Y, Sun F, Chen M, Zhou L, Wang N, et al. Adverse events associated with the treatment of multidrug-resistant tuberculosis: a systematic review and meta-analysis. Am J Ther. 2016;23(2):e521–30. https://doi.org/10.1097/01.mjt.0000433951.09030.5a.
121. Hwang TJ, Dotsenko S, Jafarov A, Weyer K, Falzon D, Lunte K, et al. Safety and availability of clofazimine in the treatment of multidrug and extensively drug-resistant tuberculosis: analysis of published guidance and meta-analysis of cohort studies. BMJ Open. 2014;4(1):e004143. https://doi.org/10.1136/bmjopen-2013-004143.
122. Gopal M, Padayatchi N, Metcalfe JZ, O'Donnell MR. Systematic review of clofazimine for the treatment of drug-resistant tuberculosis. Int J Tuberc Lung Dis. 2013;17(8):1001–7. https://doi.org/10.5588/ijtld.12.0144.
123. Hwang TJ, Wares DF, Jafarov A, Jakubowiak W, Nunn P, Keshavjee S. Safety of cycloserine and terizidone for the treatment of drug-resistant tuberculosis: a meta-analysis. Int J Tuberc Lung Dis. 2013;17(10):1257–66. https://doi.org/10.5588/ijtld.12.0863.

124. Zhang X, Falagas ME, Vardakas KZ, Wang R, Qin R, Wang J, et al. Systematic review and meta-analysis of the efficacy and safety of therapy with linezolid containing regimens in the treatment of multidrug-resistant and extensively drug-resistant tuberculosis. J Thorac Dis. 2015;7(4):603–15. https://doi.org/10.3978/j.issn.2072-1439.2015.03.10.
125. Agyeman AA, Ofori-Asenso R. Efficacy and safety profile of linezolid in the treatment of multidrug-resistant (MDR) and extensively drug-resistant (XDR) tuberculosis: a systematic review and meta-analysis. Ann Clin Microbiol Antimicrob. 2016;15(1):41. https://doi.org/10.1186/s12941-016-0156-y.
126. Sotgiu G, D'Ambrosio L, Centis R, Tiberi S, Esposito S, Dore S, et al. Carbapenems to treat multidrug and extensively drug-resistant tuberculosis: a systematic review. Int J Mol Sci. 2016;17(3):373. https://doi.org/10.3390/ijms17030373.
127. Hewison C, Khan U, Bastard M, Lachenal N, Coutisson S, Osso E, et al. Safety of treatment regimens containing bedaquiline and delamanid in the endTB cohort. Clin Infect Dis. 2022; https://doi.org/10.1093/cid/ciac019.
128. Marks SM, Mase SR, Morris SB. Systematic review, meta-analysis, and cost-effectiveness of treatment of latent tuberculosis to reduce progression to multidrug-resistant tuberculosis. Clin Infect Dis. 2017;64(12):1670–7. https://doi.org/10.1093/cid/cix208.
129. Fox GJ, Oxlade O, Menzies D. Fluoroquinolone therapy for the prevention of multidrug-resistant tuberculosis in contacts. A cost-effectiveness analysis. Am J Respir Crit Care Med. 2015;192(2):229–37. https://doi.org/10.1164/rccm.201501-0069OC.

Chapter 8
The Role of Surgical Interventions for Pulmonary TB: What Is the Role of Surgical Intervention in Pulmonary TB?

Takayuki Nakagawa

Abstract Prior to the induction of effective chemotherapy, the treatment for pulmonary TB was limited and mainly surgical. The development of anti-TB drugs has rendered TB a curable disease via chemotherapy, and the role of surgery for TB has diminished. However, in the 1990s, multidrug-resistant pulmonary tuberculosis (MDR-TB) and extensive drug-resistant tuberculosis (XDR-TB) emerged, and surgical treatment became a necessary intervention once again.

The current role of surgical treatment for pulmonary TB is to increase the treatment success rate. Surgery is indicated for drug-resistant pulmonary TB that is difficult to treat with chemotherapy alone. For patients who are persistently sputa-positive despite adequate chemotherapy, the goal of surgery is to convert the patient to sputa-negative and improve the effects of medical treatment by removing cavitary lesions and damaged lung tissues that contain a large number of TB bacilli. Surgery should also be considered for patients with large residual cavities who have a high risk of relapse, even if sputum cultures become negative after chemotherapy.

Surgical treatment for pulmonary TB consists mainly of anatomical lung resection and requires advanced surgical skills, as the dissection of adhesions on the chest wall and the isolation of hilar structures are required. In addition, various techniques for preventing bronchial stump fistulas and chest space complications are required; therefore, this surgery should be conducted by experienced surgical teams in specialized facilities. Surgery is a multidisciplinary strategy for the treatment of drug-resistant TB, and cooperation between physicians and surgeons is essential to attain the best outcomes in pre- and postoperative management and chemotherapy.

Compared with chemotherapy alone for MDR-TB and XDR-TB, adjunctive surgery combined with chemotherapy has been reported to have a higher treatment success rate. Although the significance of surgical treatment for drug-resistant TB

T. Nakagawa (✉)
Department of Thoracic Surgery, National Hospital Organization Ibarakihigashi National Hospital, Tokai, Naka-gun Ibaraki, Japan

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has not yet been clearly established, several published reports have demonstrated the effectiveness of surgical interventions for patients with pulmonary TB.

Keywords Surgical pulmonary resection · Drug-resistant TB

1 Introduction

The field of thoracic surgery was developed based on the surgical treatment for pulmonary TB. In the sanatorium era, surgical treatment primarily focused on controlling the TB cavity. Pulmonary collapse surgeries including artificial pneumothorax, thoracoplasty, phrenic nerve interruption, and prombage were developed as methods of collapsing the TB cavity. These procedures aimed to deprive *M. tuberculosis* of aerobic conditions. In the early twentieth century, an artificial pneumothorax was achieved by artificially injecting air into the chest space to contain the bacilli inside the cavity. Although artificial pneumothorax has become a common procedure, its efficacy is limited for TB cavities of the upper lung with adhesions. Thoracoplasty, in which multiple ribs are removed to reduce the rib cage volume, was used to treat patients in whom an artificial pneumothorax was not successful. Prombage was performed in patients with limited cardiopulmonary function. The costal periosteum was dissected from the ribs and the resulting space was filled with various materials. Cavitary suction or cavernostomies were also attempted to directly drain the TB cavity. As chemotherapy regimens were developed, surgical treatments for TB were gradually discontinued due to the accompanying loss of lung function and complications such as empyema, perforation of the cavity, and material infection. However, knowledge of these historical procedures provides valuable guidance regarding modern surgical treatments for pulmonary TB, as the goal of controlling the chest space after resection remains the same.

With the development of safe perioperative management, resectional TB surgery has become the standard and most effective surgical management strategy. Since the mid-twentieth century, several anti-TB drugs have been developed. Initially, surgical treatment remained the standard of care, and medical treatment was considered an adjunct therapy. However, with the introduction of rifampicin in 1963, chemotherapy alone could be used to cure TB, rendering the surgical treatment of pulmonary TB unnecessary.

In the 1990s, multidrug-resistant tuberculosis (MDR-TB), resistant to at least isoniazid and rifampicin, and extensive drug-resistant tuberculosis (XDR-TB), resistant to at least isoniazid, rifampicin, fluoroquinolones, and secondary injectable drugs emerged and became a global problem. Compared to treatment for drug-susceptible TB, chemotherapy for drug-resistant TB is complicated, not always available, requires a longer treatment period and more toxic medications, and has a high relapse rate. Patients with MDR-TB or XDR-TB require treatment from an experienced team. Patients with a large number of bacilli in TB cavities and significant lung damage cannot be successfully treated with limited chemotherapy. The success rate of chemotherapy for patients with MDR-TB is 62% [1] and that for

patients with XDR-TB is less than 43% [2]. Satisfactory treatment outcomes for these patients have not been obtained with chemotherapy alone. Therefore, in addition to chemotherapy, surgery to remove the major disease focus has been reintroduced. Surgical treatment is the shortest and best way to eliminate bacteria via the removal of TB foci, resulting in a favorable prognosis for otherwise difficult-to-treat patients.

The World Health Organization (WHO) has established guidelines regarding the indications for the surgical treatment of patients with pulmonary TB [3]. Surgery is mainly indicated for patients with MDR-TB or XDR-TB, as these types of TB are difficult to cure with chemotherapy alone. The patient's clinical condition must be considered, and the resectional range, safety, and tolerability must be evaluated preoperatively. Poor prognostic factors and contraindications for surgery must be ruled out prior to surgery.

Along with surgical treatment, individualized chemotherapy should be conducted after drug-susceptibility testing (DST). Surgery with the minimum amount of bacteria results in fewer preoperative complications. The key to successful TB treatment is the cooperation of expert physicians and skilled surgeons to develop an individualized strategy for each patient. While surgical treatment plays an adjunctive role in the treatment of pulmonary TB, it is also an indispensable treatment method for patients with difficult-to-treat TB. The efficacy of surgical treatment for pulmonary TB has been demonstrated by several studies and will be discussed in this chapter.

2 Indications for Surgical Treatment

The indications and timing of surgery for patients with pulmonary TB were proposed by the WHO in 2014 based on the results of several studies [3]. The first absolute indication is a positive sputum culture despite adequate anti-TB chemotherapy for 4–6 months. This indication is typically found in patients with MDR-TB or XDR-TB with limited effective chemotherapy options and cavitary lesions or irreversibly damaged lung tissues. The TB cavity may contain a large number of organisms (10^7–10^9), which limits the efficacy of chemotherapy drugs. The elimination of these focal burdens of bacilli is expected to convert the sputum culture to negative, prevent the progression of TB to the surrounding lung tissues, and promote the control of scattered tiny lesions.

The second indication for the surgical management of pulmonary TB is a high risk of relapse, even after a negative sputum culture is achieved via chemotherapy. Risk factors for relapse in patients with drug-resistant TB include the presence of large cavities after chemotherapy, damaged lung tissue in which TB bacteria can remain, and insufficient chemotherapy (due to allergies, toxicities, or poor immunological conditions). Positive specimens have been found in the resected lung in patients with drug-resistant TB, regardless of negative sputum cultures at the time of surgery. The management of patients with drug-resistant TB whose sputum culture relapses to positive is limited by a lack of effective medications.

For the first two indications, surgical treatment should be performed based on the aggressive regimens suggested by the results of the DST. Surgery remains an adjunctive therapy and does not replace chemotherapy. The extent of TB lesions is one of the most important factors for determining the surgical indications and resectional lung range. When the main focus lesion, such as the cavity and damaged lung tissue, is localized on one side of the lung, the surgical conditions are more favorable. When the cavitary lesions are located in both lungs, surgery is indicated if the lesions are localized to a resectable area, depending on the patient's tolerance. However, scattered nodular or infiltrative bilateral lesions are typically not resectable. Surgery serves as a debulking procedure, and the remaining lesions are treated using chemotherapy. Resectional surgery for pulmonary TB is an adjunctive procedure to be performed electively to help control the disease following chemotherapy. Tuberculomas alone is not an indication for surgery, though surgery may be performed to distinguish tuberculomas from malignant diseases such as lung cancer, or to allow for the diagnosis of TB.

Surgery is contraindicated in patients with widespread, bilateral cavitary lesions, or damaged lung tissues. Active bronchial TB should also be ruled out preoperatively. In addition, sufficient cardiopulmonary reserve is required for the surgical management of pulmonary TB. A forced expiratory volume in 1 s ($FEV_{1.0}$) less than 1.5 L for lobectomy and less than 2.0 L for pneumonectomy are contraindications for resectional surgery. However, the assessment of respiratory function affected by TB over time is not simple. Severely damaged lungs have reduced pulmonary blood flow. As a result, the contralateral lung may compensate for reduced function. Patients with pulmonary-heart failure, pulmonary hypertension, or extremely low prosperity (less than 40–50% of the normal BMI) have a poor prognosis and likely cannot tolerate surgery.

Serious complications, such as uncontrollable hemoptysis, are the third indication for the surgical management of pulmonary TB. Emergency or urgent surgery is sometimes required. However, surgery with insufficient chemotherapy should be avoided as much as possible due to perioperative complications. The timing of surgical treatment should be carefully determined based on the acute phase of recovery from bronchial artery embolization for hemoptysis and chemotherapy. However, emergency surgery is sometimes required for life-threatening massive hemorrhages, and it should be performed very selectively to avoid morbidity and mortality.

The final indication for surgery for pulmonary TB is complications and sequelae of the natural TB disease process. There are various complications and sequelae associated with TB, including pneumothorax, pyopneumothorax, pleural empyema with or without bronchopleural fistula, broncholith, post-TB stenosis of the trachea, and post-TB chronic pulmonary aspergilloma. These complications and sequelae require various procedures. Post-TB chronic pulmonary aspergillosis that infects residual structural lesions after TB treatment is a relatively common and intractable sequela of TB. Surgical resection of the affected lung is the treatment of choice, though an additional thoracoplasty or cavernostomy may be required. Even after effective chemotherapy, any remaining cavities and damaged lung tissues are an ideal environment for future infections and should be monitored carefully.

3 Preoperative Management

Preoperative management is essential for a successful surgery. For patients undergoing surgery for pulmonary TB, preoperative management includes adequate chemotherapy, appropriate timing of the surgery, and careful patient selection. Experienced physicians and thoracic surgeons must work together to develop multidisciplinary strategies for all stages of TB treatment. Risk and benefit assessments are also important prior to surgery. The long-term benefits of surgical interventions must be determined, and the risks of perioperative complications must be considered. Patients with risk factors for a poor prognosis and contraindications must not undergo surgery. Each patient's indications for surgery should be determined with reference to previously reported treatment success rates of adjunctive surgery for MDR-TB and XDR-TB compared to chemotherapy alone.

The preoperative chemotherapy regimen should be based on the DST results. Effective treatment regimens for patients with MDR-TB or XDR-TB must contain at least five drugs, and require patient compliance and adherence for at least 4–6 months. The ideal goal of chemotherapy is to obtain the lowest levels of TB and a negative sputum culture prior to surgery. Patients' sputa cultures should be assessed regularly throughout preoperative chemotherapy to evaluate the response to chemotherapy and predict the prognosis and complications of surgery.

A patient whose main lesion is confined to one lung with sufficient lung function remaining after the planned resection is a good candidate for pulmonary TB surgery. Patients who have TB lesions in both lungs have limited surgical treatment options. Limited bilateral resections or resection in the worse lung may be considered, though the indications for surgery are more limited in these patients. The bacterial count and radiological progression or improvement are assessed during chemotherapy to determine the surgical indication and resection range. Surgical interventions are contraindicated when the patient's TB cavitary lesion progresses to areas in both lungs. In addition, the patient's lung function or nutritional status may be insufficient to tolerate surgery. For patients with drug-resistant TB with progressive cavities or damaged lung tissue, it is important to administer chemotherapy while considering the timing of surgical interventions so that the appropriate window for an effective surgery is not missed.

4 Preoperative Assessments for Pulmonary Resection

The indication for surgery, resectional range of TB, and tolerance for surgery and general anesthesia must be evaluated preoperatively. The preoperative assessments for pulmonary TB surgery are similar to those for lung cancer surgery. Patients facing pulmonary TB surgery may have diabetes mellitus, weakened immunity, malnutrition, or organ damage caused by chemotherapy.

Routine blood examinations including a blood count, biochemistry test, and coagulation tests, should be conducted to evaluate the patient's inflammatory and nutritional status and to confirm the hematological toxicity, liver dysfunction, and renal dysfunction that may have been caused by anti-TB drugs.

Electrocardiography and echocardiography exams should be performed to evaluate the cardiac function and to predict the cardiac load after lung resection to avoid pulmonary hypertension and heart failure.

A spirometry pulmonary function examination is necessary to determine the surgical indications for lung resection. Although there is no absolute index for the respiratory function test, the British Thoracic Society [4] and American College of Chest Physicians [5] guidelines indicate that an $FEV_{1.0}$ greater than 1.5 L is needed for a lobectomy and an $FEV_{1.0}$ greater than 2.0 L is needed for a pneumonectomy. In patients with lower $FEV_{1.0}$, the postoperative $FEV_{1.0}$ (%ppo$FEV_{1.0}$) can be predicted using pulmonary ventilation and blood flow scintigraphy. A %ppo$FEV_{1.0}$ of 40% or more and a predicted postoperative diffusing capacity for carbon monoxide (%ppoDLco) of 40% or more are required for safe pulmonary surgery.

Lung scintigraphy is used to measure the blood and air supply to the lungs [6]. The ventilation/blood flow ratio is compared between the affected and healthy sides for the evaluation of the remaining respiratory function after surgery as well as one lung ventilation during surgery. In patients with TB, the affected lung is often decreased in size while the healthy lung is dilated and asymmetrically deformed as a result of long-term inflammation and healing. In patients with extensively damaged lung tissues, blood flow to the affected lungs will be significantly reduced, resulting in a minimal loss of respiratory function after surgery in some patients (Fig. 8.1). Lung scintigraphy is useful for an accurate prediction of postoperative

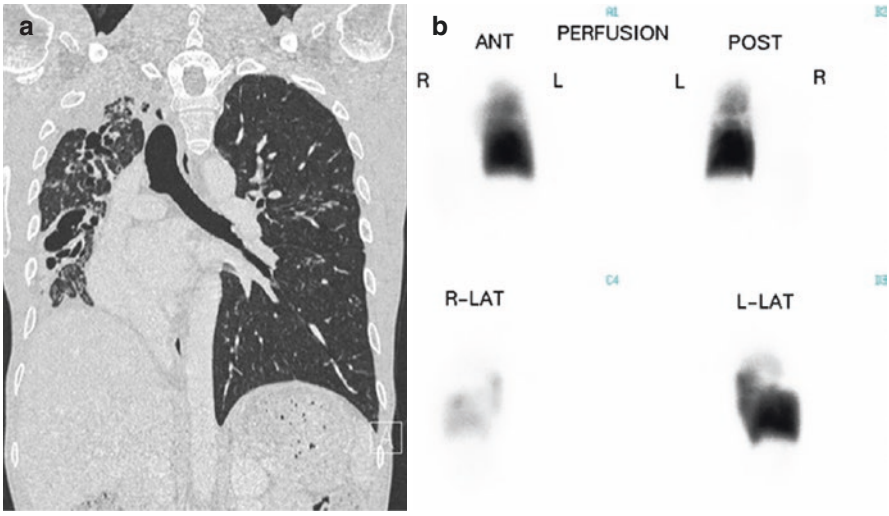

Fig. 8.1 (**a**) A computed tomography image after a right pneumonectomy in a patient with extensive drug-resistant tuberculosis (XDR-TB) is shown. (**b**): A preoperative pulmonary scintigraphy reveals an extremely low blood flow ratio to the right lung (3.4%)

lung function in patients with low pulmonary function and in those with a large resection range, such as patients who require a bi-lobectomy or a pneumonectomy.

Chest radiography and computed tomography (CT) are essential preoperative examinations. Chest CT images allow for the assessment of the extent of TB and the resectional range and to determine the surgical indications. Lungs with TB can have significantly abnormal anatomical structures. Deformations of the interlobar, bronchus, and pulmonary vessels (pulmonary artery and vein) should be examined. In addition to transverse CT images, reconstructions of sagittal and coronal images or enhanced 3-dimensional-CT images are useful for recognizing the anatomical structures of the lung preoperatively (Fig. 8.2). Inflammation of TB can extend to the chest wall and hilar region, resulting in dense pleuropulmonary adhesions and fibrotic changes in the hilar region. The preoperative understanding of a patient's unique anatomy is useful when planning the surgical approach and dissection of adhesions.

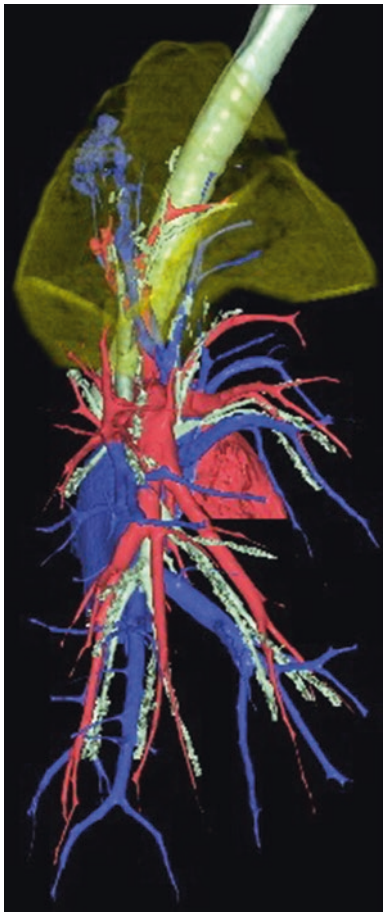

Fig. 8.2 A three-dimensional computed tomography (3D-CT) image suggesting an upper right lobectomy in a patient with pulmonary TB with repeated pneumothorax is shown. The 3D-CT reveals that the affected upper lobe is greatly deformed and the interlobar and pulmonary vessels are displaced

Bronchoscopy should also be performed before pulmonary TB surgery. Patients with bronchial TB or TB lesions in close proximity to the central bronchus may have abnormalities in the bronchial mucosa, bronchial deformity, stenosis, or obstructions. Bronchial TB lesions extending to the bronchial stump increase the risk of bronchial stump fistulas and result in a poor prognosis. Preoperative bronchoscopy may help prevent such complications via the use of an adequate flap for bronchial stump covering. Preoperative visualization of the tracheal bronchus may also help surgeons rule out unexpected malignancies and avoid complications during endotracheal intubation for general anesthesia.

A careful preoperative evaluation of the patient and the TB status is important as it reduces the risk of intraoperative and postoperative complications, which affects the patient's prognosis and surgical outcomes.

5 Surgical Procedure

Before the development of chemotherapy regimens for TB, the main surgical treatment for pulmonary TB was collapse therapy, which included artificial pneumothorax and thoracoplasty. Over the years, lung resection surgeries have become the standard surgical treatment for pulmonary TB due to improvements in anesthesia techniques, fluid therapy, and perioperative methods. However, complications such as bronchial fistulas and empyemas were frequent. With the development of chemotherapy for TB, lung resections have become relatively safe procedures. When chemotherapy emerged as a curative treatment for TB in the late-twentieth century, lung resection surgeries were performed significantly less frequently. The emergence of MDR-TB and XDR-TB has reintroduced the need for surgery in patients with pulmonary TB. Resection surgery performed to treat TB requires different techniques and knowledge than surgery for malignant diseases; therefore, the surgical treatment of a patient with TB is challenging for surgeons who lack experience.

Lung resection is the current standard surgical treatment for patients with pulmonary TB as it is an effective method for achieving a negative sputum culture in these patients. Surgery is often required to distinguish TB from malignant tumors, though this chapter focuses on pulmonary resection for the purpose of treating pulmonary TB, not on diagnostic procedures.

5.1 The Resectional Range of the Lungs

There are four basic types of resection surgery: wedge resection, segmentectomy, lobectomy, and pneumonectomy. These basic types may also be combined. Anatomical resections include segmentectomy, lobectomy, and pneumonectomy, and are the preferred procedures depending on the extent of the TB lesion. When determining the resectional range, the location of the cavitary lesions, resection margin, and safety of vessel dissection must be considered. In addition, it is

necessary to select a range of lung resection and surgical methods based on the residual chest space, which will be described later.

Wedge resection is not a type of anatomical resection, and is a relatively simple procedure. Small lesions localized just below the visceral pleura may be resected via this method. However, nonanatomical resections may result in unrecognized TB foci being included in the stapler line, which is the margin of the wedge resection. Prolonged air leak from pulmonary fistula on stapler line by entrapped TB is associated with complications such as chest space contamination. When TB lesions are included in the stapler line, there is a risk of TB reactivation. The surgeon must palpate the excision margin to confirm the absence of TB foci before stapling. A wedge resection is a surgical option when TB lesions are located in more than one lobe and the resected area needs to be minimized to preserve function due to a combined resection. However, the recommendations and guidelines for this procedure are unclear.

Segmentectomy is an anatomical resection that is useful for the preservation of lung function. However, in clinical practice for TB surgery, a segmentectomy is rarely performed alone; the procedure is often combined with a lobectomy. A left upper division segmentectomy or lingular segmentectomy is indicated for patients with pulmonary TB with a limited range of small cavities. It is necessary to identify the intersegmental plane and the vein on the segment, which is somewhat challenging. Intersegment borders are found via the ventilation method or indocyanine green imaging. Although the use of various types of segmentectomy or subsegmentectomy has reported for lung cancer surgeries, not all of these types of surgeries can be applied to patients with pulmonary TB as the pulmonary anatomical structure and vasculature are significantly deformed due to inflammation in patients with TB, and a complex demarcation zone in which the TB cavity is close to the stapler line may lead to peripheral bronchial fistulas or pulmonary fistulas, similar to the fistulas that must be avoided during wedge resections. An uncomplicated surgery is the first priority, though preserving lung function is important for the patient's long-term prognosis.

The most frequently performed surgery for pulmonary TB is the lobectomy, which is indicated for patients with a TB cavity in a single lung lobe. Approximately 40–70% of the reported resections for patients with MDR-TB or XDR-TB are lobectomies [7–13]. This anatomical resection is commonly performed by surgeons as it is the standard procedure for lung malignancies. As with segmentectomy, the interlobar formation and pulmonary vessel dissection require careful techniques as the lung structures and have undergone sclerotic changes due to TB inflammation.

Combined resections, such as a bi-lobectomy or a lobectomy plus a segmentectomy are used when the patient has TB cavities in more than one lung lobe. A combined resection should be removed as a single block without separating the interlobar lung. Surgeons may need to access or dissect structures in the hilar region, such as pulmonary vessels and bronchi. A combined resection may eliminate the need for a pneumonectomy. The volume of residual lung must be considered when planning combined procedures. Combined resection leaves more chest space than a single lobectomy, the reduction of which requires additional procedures.

A pneumonectomy is indicated in patients who have damaged lung tissue throughout one entire lung. If chemotherapy is not effective to prevent the

progression of TB from the affected lung to the healthy lung, the patient may not be a surgical candidate. Pneumonectomy is often the last treatment option for patients with drug-resistant TB that is difficult to treat. However, pneumonectomy is a high-risk procedure with high mortality and morbidity rates. Nevertheless, pneumonectomy for MDR-TB/XDR-Tb is performed in about 20–60% in practice [7–13]. A pneumonectomy is a simple procedure compared with other anatomical resections as the pulmonary vessels and main bronchus are dissected without separating the hilar or interlobar elements once the adhesions are removed from the chest wall. However, a pneumonectomy is indicated in very few patients due to the risks of post-pneumonectomy syndrome, respiratory failure, cardiovascular failure, and pleural space complications, which affect not only the perioperative period but the rest of the patient's life. However, the pre- and intraoperative blood flow to the affected side is expected to be significantly reduced due to chronic inflammation, and the condition of the healthy lung and the postoperative shift in lung function are key factors determining a patient's tolerance to a pneumonectomy.

5.2 Pitfalls of the Surgical Procedure

The inflammation caused by TB typically results in adhesions between the chest wall and lungs. Adhesions may be limited and mild; however, extensive TB lesions lead to widespread adhesions that are difficult to remove. When the patient has chronic inflammation or a coinfection of *Aspergillus*, the adhesions become denser and develop into chest wall vessels. The dissection of adhesions takes a lot of time, results in blood loss, and leads to difficulties in the apex region or the diaphragm. TB cavities are often found adjacent to the surface of the lung that has adhered to the chest wall, and the lung and pleura must be carefully dissected to avoid exposing the contents of the lung cavity. Extrapleural dissection may be required in patients with dense adhesions.

The careful isolation of the hilar structures is necessary. Inflammatory, swollen lymph nodes or calcified lymph nodes in the hilar region lead to difficulties during the dissection of the pulmonary vessels. However, the dissection of the hilar lymph nodes is not necessary, unlike in malignant tumor surgery. Lymph nodes are removed only for the separation of hilar structures. When the lymph nodes are tightly packed, the dissection requires careful techniques.

5.3 Prevention of Complications

The perioperative morbidity rates of resectional TB surgery have been reported as 12–25% and the mortality rates have been reported as 0–3.3% [7–13]. Major complications of resectional TB surgery include bronchopleural fistula (BPF) and chest space complications. BPF leads to empyema, a relapse of TB, and a poor prognosis. Bronchial stumps must be reinforced to prevent BPF. The most common coverings for the bronchial stump are the pericardial fat pad or an intercostal muscle flap,

while the latissimus dorsi muscle is sometimes used [11]. Effective chemotherapy is also important to control the remaining TB bacilli near the bronchial stump at the time of surgery, and a negative sputum culture is preferred.

After lung resection, the chest space is gradually narrowed due to expansion and movement of the residual lung, leading to a mediastinal shift and deformation of the chest wall. When the post resectional space is large, as in patients undergoing pneumonectomy, bi-lobectomy, or lobectomy in which the residual lung is not sufficiently shifted and fills the space due to adhesions, additional procedures are required to avoid chest space complications. Thoracoplasty is effective for the elimination of the residual space in these patients (Fig. 8.3).

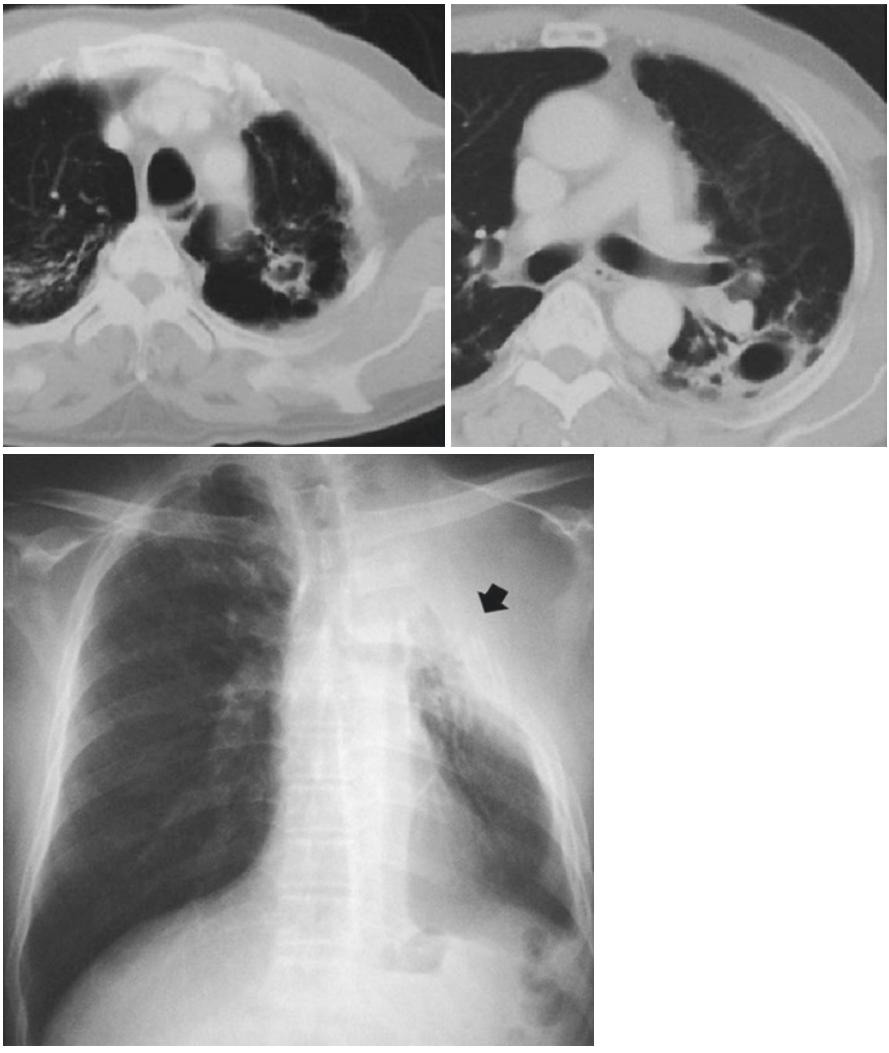

Fig. 8.3 Images of a patient with multidrug-resistant TB who underwent a combined left upper lobectomy and S6 segmentectomy and a limited thoracoplasty are shown. The thoracoplasty was performed to collapse the chest space after the lobectomy and segmentectomy

5.4 The Surgical Approach for Lung Resections

With the development of surgical devices, lung resection for cancer has become less invasive over the past 30 years. The surgical approach has changed from an open thoracotomy to video-assisted thoracoscopy (VATS) to robotic-assisted thoracoscopic surgery (RATS). The surgical approach for pulmonary TB is no exception; minimally invasive surgeries are preferred. However, minimally invasive surgery is not as common for patients with pulmonary TB. Thoracotomies for pulmonary TB are often conducted via a posterior lateral incision or an anterior incision. Less invasive approaches result in less damage to the chest wall, an earlier recovery after surgery, and earlier rehabilitation. While the surgical approach depends greatly on the experience of the facility, the surgical team, and the skill level of each surgeon, minimally invasive surgery for pulmonary TB is challenging for several reasons.

Resectional TB surgery requires more careful dissection of adhesions, and a minimally invasive approach may limit the dissection of dense adhesions. Extrapleural dissections are required for patients with dense adhesions. Inflammation caused by TB also affects the hilar structures and perivascular areas. A failed hilar vascular dissection may result in fatal bleeding. Minimally invasive surgery can lead to longer surgery times and more bleeding in some patients. Additional procedures for bronchial stump coverings or chest space complications are also more difficult using a minimally invasive approach. Minimally invasive access is not the ultimate goal for pulmonary TB surgery. The surgical approach and procedure that will result in the most successful treatment should be used.

6 Postoperative Management and Chemotherapy

The perioperative management of patients with pulmonary TB is similar to that of patients with lung malignancies. The patient's respiratory and circulatory dynamics, chest tube drainage, and pain should be managed in an intensive care unit. The appropriate analgesics should be administered, including opioids. After confirming the expansion of the residual lungs and the condition of the chest space via chest radiography, physiotherapy, and respiratory exercises should be initiated. Depending on the surgical invasion, the patient should be monitored for early and late complications, including air leaks, BPFs, chest space complications, and pleural empyema.

Long-term follow-up with the physician and the surgeon is preferred, as this helps detect late complications (including residual cavity complications and bronchial stump fistulas) and provides surgeons with an opportunity to understand the postoperative outcomes.

Anti-TB chemotherapy can be resumed as the patient resumes oral intake postoperatively. Postoperative chemotherapy typically consists of the same regimens that were used preoperatively and is necessary to treat the remaining scattered nodular lesions and tiny cavities. Effective postoperative chemotherapy reduces

complications, contributing to treatment success. The duration of postoperative chemotherapy after surgery depends on the status of the patient's sputum culture and the bacteriological results of the resected lung tissue.

The timing of the conversion of the patient's sputum culture from positive to negative must be considered when determining the duration of postoperative chemotherapy. Although the optimal duration of postoperative anti-TB chemotherapy is not clearly defined, the following recommendations based on previous studies and guidelines provided by the WHO [3] can be used. For patients with culture-positive TB at the time of surgery, postoperative chemotherapy should be conducted for 4–6 months for patients with susceptible TB, 18 months for those with MDR-TB, and 24 months for those with XDR-TB after culture conversion. For culture-negative patients at the time of surgery, at least 4 months of postoperative chemotherapy is recommended for patients with susceptible TB and 6–8 months of postoperative chemotherapy is recommended for patients with MDR-TB or XDR-TB. However, the physician should consider the clinical condition of each individual patient based on the available anti-TB chemotherapy regimens, the remaining TB lesions, and the patient's comorbidities.

The bacteriological results of the resected lung tissue that convey the presence of TB bacilli in the lung cavities must also be considered when determining the duration of postoperative chemotherapy. These results are also important when determining the success of preoperative chemotherapy. However, the adjustment of anti-TB chemotherapy based on the bacteriological examination of the surgical specimen has not been sufficiently evaluated, and further research is needed.

The success of the TB treatment depends not only on the surgical procedures but also on patient management and effective postoperative chemotherapy. The cooperation of specialists and surgeons is essential.

7 Surgical Treatment Outcomes

With the global emergence of MDR-TB and XDR-TB, several studies regarding surgical resection have reported the efficacy and safety of surgical interventions for pulmonary TB. Most of these studies were conducted in Asia or North America. As the social system and medical resources are significantly different across the world, it is difficult to directly compare the results of these studies. However, positive outcomes have been reported.

Iseman et al. performed lung resection surgeries in 29 patients with drug-resistant pulmonary TB from 1983 to 1988 with a treatment success rate of 92%, no operative deaths, and a morbidity rate of 6.8%. These results are considerably more favorable than the reported success rate of chemotherapy alone for drug-resistant TB of 56% and suggest that surgery is effective for patients with resectable or localized MDR-TB with a high possibility of failure or relapse [14].

Pomerantz et al. performed 180 pulmonary resections in 172 patients with drug-resistant pulmonary TB from 1983 to 2000. While this study is a retrospective

cohort study, it has included the largest sample size to date. The treatment success rate exceeded 90%, with an operative mortality rate of 3.3%, and a significant morbidity rate of 12%. The authors of this study recommended the use of muscle flaps to prevent complications [7].

Shiraishi et al. reported a case series of 30 lung resection surgeries for patients with MDR-TB from 2000 to 2002. While there was no operative mortality in this study, the complication rate reached 30% and included BPF with empyema, chest space complications, and prolonged air leaks. In a later study, the authors reported a lower complication rate when individualized multidrug chemotherapy and additional procedures were performed. The authors used the latissimus dorsi muscle to cover the bronchial stump and as a muscle tent over the raw residual lung tissue. A limited thoracoplasty was performed to reduce chest space complications. The authors emphasized the importance of pre-and postoperative chemotherapy and the importance of monitoring patients for bronchial stump and chest space complications [12, 15].

Since 2000, several studies have reported the surgical treatment of drug-resistant pulmonary TB, and several systematic reviews and meta-analyses have been conducted to analyze the effectiveness of surgery in these patients. In a meta-analysis including 15 studies and 949 patients with MDR-TB or XDR-TB, Xu et al. estimated the pooled treatment success rate to be 84%, with a failure rate of 6%, a relapse rate of 3%, a mortality rate of 5%, and a default (treatment interruption) rate of 3%. Although resectional TB is an adjunct surgery, the authors concluded that resectional TB surgery results in better outcomes than medical treatment [16]. Rebecca et al. performed a meta-analysis of 14 surgical versus nonsurgical studies regarding the effectiveness of surgical treatment. According to this analysis, the treatment success rate was 81.9% in the surgical group and 59.7% in the nonsurgical group (odds ratio = 2.62). Adjunctive surgery after chemotherapy was more effective than chemotherapy alone [17].

The high success rates of adjunctive surgery for patients with MDR-TB or XDR-TB reported in these studies suggest that combination therapy is more effective than chemotherapy alone. However, the previous reports were based on prospective or retrospective cohort studies, and no randomized controlled trials have been conducted, resulting in low evidence levels. Future research is needed to obtain more reliable evidence.

The more favorable outcomes achieved by surgical interventions are dependent on the appropriate surgical indications and effective chemotherapy. In many developed countries, individualized chemotherapy is performed using the most aggressive regimen based on the DST results. In recent years, new anti-TB drugs such as linezolid, bedaquiline, and delamanid have been developed and are expected to cure MDR-TB and XDR-TB. These new drugs are likely to change the role of surgical treatment in the near future. However, the outcomes of surgical treatment for pulmonary TB are also favorable in developing countries with limited medical resources [18, 19]. Furthermore, appropriate surgical treatment can lead to reduced treatment costs [9]. Even as novel drugs are introduced, surgery will continue to play a role in the treatment of pulmonary TB.

8 Conclusion

Surgery plays an important role in the multidisciplinary treatment of pulmonary TB, especially in patients with MDR-TB and XDR-TB. In patients with positive sputum cultures despite effective chemotherapy, the surgical resection of the cavities and damaged lung tissues leads to favorable outcomes. Even in patients who achieve negative sputum cultures with chemotherapy, the removal of cavitary lesions is expected to reduce the risk of relapse. Surgery is indicated in patients with TB lesions localized to one lung that are within the resectable range. Individualized chemotherapy based on DST results is necessary pre- and postoperatively. Appropriate preoperative management, evaluation of surgical tolerance, postoperative follow-up, and cooperation between physicians and surgeons are necessary. Resectional TB surgery should be performed by surgeons who have experience dissecting adhesions to reduce complications. The combination treatment of chemotherapy and surgery has achieved a success rate of approximately 80–90% in patients with drug-resistant TB. However, more research is required to verify the role of surgery for patients with pulmonary TB.

References

1. Orenstein EW, Basu S, Shah NS, et al. Treatment outcomes among patients with multidrug-resistant tuberculosis: systematic review and meta-analysis. Lancet Infect Dis. 2009;9:153–61.
2. Migliori GB, Sotgiu G, Gandhi NR, et al. Drug resistance beyond XDR-TB: results from a large individual patient data meta-analysis. Eur Respir J. 2013;42:169–79.
3. World Health Organization. The role of surgery in the treatment of pulmonary TB and multidrug- and extensively drug-resistant TB. WHO; 2014. p. 1–23.
4. British Thoracic Society and Society of Cardiothoracic Surgeons of Great Britain and Ireland, Society of Cardiothoracic Surgeons of Great Britain and Ireland Working Party. Working Party. BTS guidelines: guidelines on the selection of patients with lung cancer for surgery. Thorax. 2001;56:89–108.
5. Datta D, Lahiri B. Preoperative evaluation of patients undergoing lung resection surgery. Chest. 2003;123:2096–103.
6. Cukic V. Preoperative prediction of lung function in pneumonectomy by spirometry and lung perfusion scintigraphy. Acta Inform Med. 2012;20:221–5.
7. Pomerantz BJ, Cleveland JC Jr, Olson HK, et al. Pulmonary resection for multi-drug resistant tuberculosis. J Thorac Cardiovasc Surg. 2001;121:448–53.
8. Chan ED, Laurel V, Strand MJ, et al. Treatment and outcome analysis of 205 patients with multidrug-resistant tuberculosis. Am J Respir Crit Care Med. 2004;169:1103–9.
9. Somocurcio JG, Sotomayor A, Shin S, et al. Surgery for patients with drug-resistant tuberculosis: report of 121 cases receiving community-based treatment in Lima, Peru. Thorax. 2007;62:416–21.
10. Van Leuven M, De Groot M, Shean KP, von Oppell UO, Willcox PA. Pulmonary resection as an adjunct in the treatment of multiple drug-resistant tuberculosis. Ann Thorac Surg. 1997;63:1368–72. discussion 1372
11. Wang HF, Lin HS, Jiang G. Pulmonary resection in the treatment of multidrug-resistant tuberculosis: a retrospective study of 56 cases. Ann Thorac Surg. 2008;86:1640–5.

12. Shiraishi Y, Katsuragi N, Kita H, et al. Aggressive surgical treatment of multidrug-resistant tuberculosis. J Thorac Cardiovasc Surg. 2009;138:1180–4.
13. Kang MW, Kim HK, Choi YS, et al. Surgical treatment for multidrug-resistant and extensive drug-resistant tuberculosis. Ann Thorac Surg. 2010;89:1597–602.
14. Iseman MD, Madsen L, Goble M, et al. Surgical intervention in the treatment of pulmonary disease caused by drug-resistant Mycobacterium tuberculosis. Am Rev Respir Dis. 1990;141:623–5.
15. Shiraishi Y, Nakajima Y, Katsuragi N, et al. Resectional surgery combined with chemotherapy remains the treatment of choice for multidrug-resistant tuberculosis. J Thorac Cardiovasc Surg. 2004;128:523–8.
16. Xu HB, Jiang RH, Li L. Pulmonary resection for patients with multidrug-resistant tuberculosis: systematic review and meta-analysis. J Antimicrob Chemother. 2011;66:1687–95.
17. Harris RC, Khan MS, Martin LJ, Allen V, Moore DA, Fielding K, Grandjean L, LSHTM MDR-TB Surgery Systematic Review Group. The effect of surgery on the outcome of treatment for multidrug-resistant tuberculosis: a systematic review and meta-analysis. BMC Infect Dis. 2016;16:262.
18. Kir A, Inci I, Torun T, et al. Adjuvant resectional surgery improves cure rates in multidrug-resistant tuberculosis. J Thorac Cardiovasc Surg. 2006;131:693–6.
19. Mohsen T, Zeid AA, Haj-Yahia S. Lobectomy or pneumonectomy for multidrug-resistant pulmonary tuberculosis can be performed with acceptable morbidity and mortality: a seven-year review of a single institution's experience. J Thorac Cardiovasc Surg. 2007;134:194–8.

Part III
Latent TB Infection: TB Prevention

Chapter 9
Advances in Diagnosis of Latent TB Infection: What Is the Latest Approach to Diagnose Latent TB Infection to Prevent TB?

David J. Horne and Asa Tapley

Abstract Both types of tests for the diagnosis of latent tuberculosis infection (LTBI), the tuberculin skin test (TST) and interferon-gamma release assays (IGRAs), rely on detecting evidence of cell-mediated immunity to *M. tuberculosis* antigens. If testing is positive and there are no symptoms, radiographic or microbiologic evidence of TB disease, the patient is typically considered to have LTBI. Advantages of the TST include low cost, ease of administration, no lab requirement, and adjustable interpretation cut-off per individual LTBI risks. Advantages of IGRAs include requiring a single visit, higher specificity compared to the TST, and likely modestly improved sensitivity (particularly with immunocompromised individuals). The TST and IGRA have only poor-to-fair concordance. Both the TST and IGRAs have significant shortcomings. They cannot discriminate asymptomatic infection from past infection, identify individuals at elevated risk of TB disease, or be used to assess response to preventative therapy. A variety of novel technologies are in different phases of investigation, development, or clinical use that may help address these issues.

Keywords Latent TB infection · Tuberculin skin test · Interferon-gamma (IFN-γ) release assay

D. J. Horne (✉)
Division of Pulmonary, Critical Care and Sleep Medicine, Department of Medicine, University of Washington, Harborview Medical Center, Seattle, WA, USA
e-mail: dhorne@uw.edu

A. Tapley
Division of Allergy and Infectious Diseases, Department of Medicine, University of Washington, Seattle, WA, USA

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1 Introduction

For the purposes of clinical and programmatic management, latent tuberculosis infection (LTBI) is defined as:

- Evidence of cell-mediated immunity to TB antigens (either a positive tuberculin skin test or interferon-gamma [IFN-γ] release assay).
- No clinical signs or symptoms of TB disease.
- No radiographic signs of TB disease.
- If specimens are collected (e.g., sputum), then cultures are negative for *M. tuberculosis*.

There is no test that is capable of directly detecting the presence of *M. tuberculosis* causing LTBI. The commercially available tests rely upon the host immune response to detect evidence of prior exposure to *M. tuberculosis*. A positive test does not distinguish between those who have had a TB exposure with T-cell priming but have cleared their infection, persons with viable TB in a controlled state ("LTBI"), or patients with TB disease. This lack of discrimination contributes to the poor performance of these tests in predicting who will progress to TB.

The tuberculin skin test (TST) was the only widely available test for LTBI diagnosis until 2001, when a commercial interferon-γ release assay (IGRA) became available: the first generation QuantiFERON-TB test (Cellestis Ltd.). After hundreds of studies evaluating the accuracy of IGRAs, it is clear that both the TST and IGRAs are acceptable but less than perfect tests [1]. In the following sections, we discuss the immunology, history, procedures, performance, strengths, and limitations of the TST and IGRAs in the diagnosis of LTBI, as well as new diagnostic approaches under investigation.

2 The Science of LTBI Testing

2.1 TB Exposure and the Immune Response

Exposure to *M. tuberculosis* occurs through the inhalation of droplet nuclei that remain airborne after being expelled by individuals with active pulmonary TB [2, 3]. Those infective droplet nuclei small enough to navigate the bronchi and avoid mucociliary clearance arrive at the terminal alveoli, where they are phagocytized by resident alveolar macrophages [4]. It is thought that some individuals clear the bacilli through innate immune defenses and tests for LTBI (TSTs or IGRAs) will remain negative due to the lack of T-cell activation.

M. tuberculosis has a number of mechanisms for avoiding lysosomal destruction after phagocytosis, allowing for persistence and replication within the macrophage until causing cell death [5, 6]. Infected macrophages release cytokines and chemokines that attract more phagocytic cells, along with neutrophils, to the site of

infection, which in turn leads to more infected cells and expansion and dissemination of the infection [7]. During this process, alveolar dendritic cells take up and transport bacilli or their remnants to regional lymph nodes and present *M. tuberculosis* antigens to naïve $CD4^+$ and $CD8^+$ T-cells, leading to their differentiation into $CD4^+$ helper T-cells type 1 and $CD8^+$ cytotoxic T-cells, respectively [8]. Once activated, these effector T-cells migrate hematogenously to the site of infection. B-cells are also activated and differentiate into specific antibody-secreting cells.

The cell-mediated immune response typically develops within a few weeks after initial infection, in which case TST and IGRAs will normally become positive [9]. The arrival of effector T-cells to the site of infection leads to activation of proximal macrophages, largely mediated by the release of IFN-γ by T-helper cells, causing morphological changes in the macrophages and enhancing their mycobacterial killing [8]. Progressively mononuclear cells aggregate and organize around the infected macrophages, extracellular bacilli, and cellular debris to form nodular structures called granulomas [10]. If cell-mediated immunity is effective, the bacilli are eliminated or contained (i.e., LTBI). If it fails—sometimes months or even years later—the bacteria proliferate and spread, the pro-inflammatory immune response causes worsening collateral tissue destruction, and subclinical disease progresses to symptomatic TB [11, 12].

It is important to note that while patients infected with *M. tuberculosis* are commonly categorized pragmatically as either having LTBI or active TB disease for public health and clinical purposes [13], the natural history of TB involves a spectrum between infection and active TB disease that is likely dynamic over time [14].

2.2 *Measuring the Immune Response to* M. tuberculosis *Exposure*

Current methods for diagnosing LTBI rely on detecting evidence of acquired immunity to *M. tuberculosis*, based on the principle that T-cells sensitized to *M. tuberculosis* antigens will predictably release cytokines when re-exposed to these antigens. In the case of the TST, purified protein derivatives from *M. tuberculosis* cultures are injected intradermally. If the person's cellular immune system has previously been exposed to *M. tuberculosis*, specific memory helper T-cells are rapidly activated by local antigen-presenting cells (e.g., dendritic cells), leading to clonal expansion and the release of IFN-γ and other pro-inflammatory cytokines [15]. The subsequent local vasodilation, edema, and infiltration of immune cells into the area near the site of the injection create a visible induration that can be measured to determine if it meets the threshold for a positive result [16].

In contrast to the TST, IGRAs are an ex vivo test to assess for *M. tuberculosis* sensitized T-cells. Depending on the specific test, whole blood or peripheral blood mononuclear cells (PBMCs) from the patient are incubated with *M. tuberculosis* antigens, leading to the release of IFN-γ by sensitized T-cells [17]. The IFN-γ

production is then measured by one of two types of enzyme-linked immunosorbent assays to measure either the total concentration of IFN-γ produced by all cells or the number of cells secreting IFN-γ. Unlike the TST, which uses a nonspecific mixture of mycobacterial proteins and is vulnerable to eliciting false-positive results due to prior BCG vaccination or environmental nontuberculous mycobacteria (NTM) exposure, IGRAs use proteins encoded by genes found in the genome of *M. tuberculosis* and other members of the TB-causing *M. tuberculosis* complex (e.g., *M. africanum*, non-BCG strains of *M. bovis*, *M. canetti*) but not shared by BCG substrains or most NTM species (exceptions include *M. flavescens*, *M. kansasii*, *M. marinum*, and *M. szulgai*) [18].

Although based on similar principles, it has been recognized since the introduction of IGRAs that there is poor-to-fair concordance with the TST. Some of the discrepancies are explained by the superior specificity of IGRAs in high-risk populations due to lack of cross-reactivity with the bacillus Calmette-Guérin (BCG) vaccine and lesser cross-reactivity to antigens from NTM. However, discordance between the tests is observed in individuals without a history of BCG vaccination.

While IGRAs require only a single visit for testing, they are more expensive to perform than the TST, require a laboratory, have a single approved cut-off for a positive result regardless of LTBI risk, and may have lesser specificity in low-risk populations [19–21]. IGRAs and the TST share similar limitations including dependence on an individual's immune system for accurate test performance, inability to differentiate between points on the TB spectrum of disease, and poor positive predictive value.

3 Tuberculin Skin Test

3.1 TST History

Robert Koch, in 1890 presented his findings on "tuberculin," a discovery that he believed would prevent and cure TB [22]. Subsequently called "Koch's Old Tuberculin," it was prepared by taking a liquid broth in which *M. tuberculosis* had been cultured, sterilized with heat, and reduced in volume through evaporation [23]. Although not of therapeutic value, in the early part of the twentieth century Clemens von Pirquet discovered that when children with TB were inoculated with Koch's Old Tuberculin, a papule would transiently appear at the site of inoculation. Mantoux's description of the intradermal injection of 0.1 ml of tuberculin, published in 1910, describes a test that is used to this day [23]. In 1934, Florence Seibert developed a process for preparing tuberculin based on steaming cultures of *M. tuberculosis* and purifying the proteins through precipitation with ammonium sulfate resulting in purified protein derivative or PPD [24]. PPD-S, for "standard," was developed for use in the USA in 1944, and the current standard US preparation is PPD-S2. Two commercially available forms of PPS-S2 are Aplisol (JPH Pharmaceuticals, Inc.) and Tubersol (Sanofi Pasteur Ltd.), differing in the methods

of protein precipitation. PPD formulations used outside of the USA include PPD RT23 produced by the Statens Serum Institute, the Japanese product PPD-s, and PPD RT23 Mexico, used in Latin America [24]. Some studies have demonstrated differences in potency between different formulations of PPD [24] and even differences between different forms of PPD-S2 [25].

3.2 Performing TST

Variability in TST results may be introduced by differences in tuberculin placement technique and should be performed by medical personnel with adequate training and experience. The preferred method for TST application is the Mantoux method, in which an intradermal injection of tuberculin is applied away from skin lesions and veins. In the USA, 0.1 ml of 5 tuberculin units of PPD-S2 are injected into the volar surface of the forearm. When performed correctly, a 6–10 mm diameter wheal (raised area of the skin) will occur. When there are concerns about TST administration, a second TST can be performed at a site at least several centimeters from the first or on the alternate forearm [26].

TSTs should be read between 48 and 72 h after placement, which is when induration is maximum. It is important to note that the size of induration (swelling), not erythema, determines a person's response. If a test is read after 72 h and meets the size criteria for a positive test, then the patient may be diagnosed with latent TB infection. However, if a test is read after 72 h and is interpreted as negative, a repeat test should be performed. The tuberculin response should be read by a trained examiner. Induration should be determined by palpation with the fingertip, marking of the borders, and measured across the forearm (transverse diameter) with the measured diameter recorded in millimeters. The tuberculin formulation and lot number, forearm used, dates of placement and reading, and size in millimeters should be recorded.

When TSTs are placed in both arms of a single person, the variation in the size of induration averages 15% [27]. Variability in interpreting tuberculin responses by a single trained reader is also about 15%, while variability between different trained readers is greater, on average 2.5 mm [28]. Patients should not be permitted to interpret their own tuberculin responses.

Multiple puncture tests (e.g., Tine and Heaf tests) were frequently used in the past due to ease of administration. However, it is difficult to control the dose of tuberculin with these tests and they are no longer recommended for use in the USA [29]. In the 1990s, the US CDC recommended the use of anergy testing in conjunction with TST in people living with HIV to assess cellular immune function. As most people have been exposed to common fungi and vaccine antigens, preparations (e.g., tetanus toxoid, mumps, or Candida antigens) were injected intradermally to assess for an intact delayed hypersensitivity response. However, this is no longer recommended as a positive delayed-type hypersensitivity response to antigens other than PPD may remain despite loss of tuberculin reactivity [26].

3.3 Safety

Local reactions at the site of tuberculin placement, including blistering and local necrosis, may occur in 1–2% of positive skin tests [30, 31]. Rarely (<0.1/1,000,000 tests), serious hypersensitivity reactions including anaphylaxis may occur due to the TST [32]. A positive TST reaction may cause long-lasting skin discoloration [8]. Patients with local or hypersensitivity reactions should be instructed to never again receive tuberculin and should instead undergo an IGRA if indicated.

3.4 TST Interpretation

TST interpretation is risk-stratified using three different sizes of induration to determine a positive response. These thresholds for positivity are based on TST accuracy in different populations, LTBI prevalence in different groups, and risk for progression to active TB [13] (see Fig. 9.1).

For persons who are subject to repeat TST testing, for example, as a workplace requirement, a TST conversion is defined as a newly positive test (based on the cut-off categories in Fig. 9.1) that is at least 10 mm larger than the previous value [29]. There is no role for repeating TSTs in patients who have been treated for LTBI in order to assess for a treatment response [33].

3.5 Accuracy

Due to the lack of tests to directly identify *M. tuberculosis* in latently infected persons, most studies of LTBI test accuracy use surrogate outcomes, usually estimating sensitivity in patients with current or prior culture-confirmed TB and specificity in persons who are at low risk for TB exposures.

The sensitivity of the TST, based on responses in healthy persons with previously treated TB is 95–98% [13]. Several factors are associated with false-negative TST results. During the period immediately following infection with *M. tuberculosis*, tuberculin responses may be negative as T-cell reactivity can take 6–10 weeks to fully develop. False-negative reactions are also more common in infants and young children, in the presence of severe illness (including extensive TB), after recent viral and bacterial infections, in immunosuppressed persons, and in persons receiving immunosuppressive drugs including corticosteroids and TNF-alpha blockers [13]. Prednisone doses of >10–15 mg/day have been associated with a depressed tuberculin response [34, 35]. One study of patients with TB and positive TST were administered 40 mg of prednisone daily and found that the mean time to TST reversion occurred on day 14 of corticosteroid treatment and that return of TST positivity occurred an average of 6 days after discontinuation of prednisone [36]. Methotrexate has not been associated with an altered tuberculin response [37].

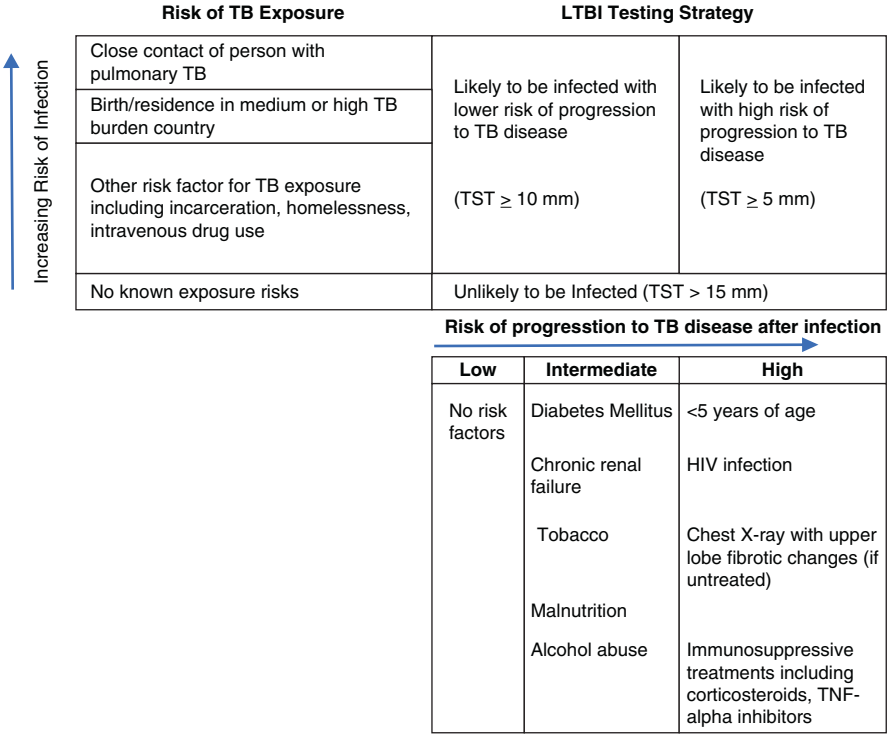

Fig. 9.1 Recommended tuberculin skin test cut-offs for diagnosing latent TB infection and rationale based on risk of infection and risk of progression to TB disease if a person is infected with *M. tuberculosis*. Adapted from Lewinsohn DM et al. Official American Thoracic Society/Infectious Diseases Society of America/Centers for Disease Control and Prevention Clinical Practice Guidelines: Diagnosis of Tuberculosis in Adults and Children. Clinical Infectious Diseases 2017;64(2):111–5

It has long been dogma that recent vaccination with live-attenuated viruses is associated with lower tuberculin responses [26]. In fact, studies have found mixed effects on TST responses after live-attenuated vaccines. Additionally, impacts on TST responses after vaccination with killed virus vaccines have also been noted. The effect of mRNA-based vaccines, such as those against SARS-CoV-2, are unknown. When possible, tuberculin testing should be performed on the same day as a vaccination or at least 4 weeks following vaccination [26].

The specificity of the TST varies by patient group, depending on the frequency of BCG vaccination and exposures to environmental NTM. TST specificity among non-US-born persons (most of whom were BCG vaccinated) has been estimated at 70%, compared to 92% for US-born persons (where BCG vaccination has never been universally recommended) [38]. The likelihood that prior BCG vaccination will cause a false-positive TST is much greater if the individual was vaccinated after age 1 year or within 8 years prior to tuberculin testing [39]. TST may be more specific than IGRAs when testing low-risk populations [19, 20], likely related to the use of risk-based cut-points to define TST positivity.

Following exposure to *M. tuberculosis* or certain other mycobacteria, the resultant hypersensitivity is typically lifelong and reflected in all future TSTs. However, it can wane over time to the point where the reaction disappears. This is more common in older adults and people whose hypersensitivity was due to BCG vaccination or NTM exposure [40]. In these cases, an initial TST may be negative, but the antigen exposure from the TST stimulates the cell-mediated immune system such that if a second TST is done within weeks, it will become positive [28]. This is called the booster phenomenon. The boosting is most often seen when the interval between TSTs is about 1–4 weeks, but the effect can last months. Because boosting can be falsely interpreted as a new *M. tuberculosis* infection, for people such as healthcare providers who will undergo TSTs regularly (as well as for some at-risk groups like residents of long-term care facilities), repeat testing in 1–4 weeks is suggested for all individuals who initially test negative in order to assess for boosted reactions and confirm the person's baseline tuberculin response [13]. Such two-step TSTs are done only once to document for future reference. Note, TSTs alone (without any prior exposure to mycobacteria) do not sensitize individuals and will not cause false-positive tests [26].

3.6 Summary

The TST has a number of advantages including >100 years of experience, no need for phlebotomy or a laboratory, low cost, differential interpretation cut-offs based on LTBI risk and risk of progression, and serial testing criteria for conversion. This is balanced against limitations that include errors in performing the TST placement, variability in assessing induration size, lower specificity due to BCG vaccination and NTM exposures, requirements for two healthcare encounters, frequent patient and/or clinician resistance to a positive finding, and poor positive predictive value.

4 Interferon-Gamma Release Assays

4.1 History of IGRAs, Discussion of QFT, T-SPOT

An assay to diagnose bovine TB, based on the detection of IFN-γ in response to a specific antigen, was developed in 1990 [41]. In 1998, a report on the performance of the QuantiFERON-TB assay, an IGRA for the detection of LTBI in humans, was published that showed high specificity and sensitivity in comparison to the TST [42]. The second generation assay, QFT-Gold (QFT-GIT), was approved in Japan and the USA in 2005. QuantiFERON-TB Gold Plus assay (QFT-Plus; Qiagen), the currently available fourth-generation QFT assay, received European approval in 2016, US approval in 2017, and Japanese approval in 2018. T-SPOT.TB, developed

in the late 1990s by Lalvani et al. [43], is manufactured by Oxford Immunotec (Abingdon, UK). T-SPOT.TB has been available in Europe since 2004, in the USA since 2008, and in Japan since 2012.

At the time of this writing, there are two commercially available IGRAs: QuantiFERON-TB Gold Plus assay and T-SPOT.TB. Like the TST, IGRAs rely on host T-cell responses to *M. tuberculosis* antigens, with prior exposure eliciting IFN-γ production. The two IGRAs differ in that T-SPOT is an enzyme-linked immunospot (ELISPOT) assay whereas QFT is enzyme-linked immunosorbent assay (ELISA) based. The result from each test is based on the spot-forming cells (SFC) or quantification of IFN-γ in international units (IU) per milliliter for T-SPOT and QFT, respectively.

4.2 Procedure and Results

4.2.1 QFT-Plus Procedure

QFT-Plus differs from prior generations of the assay in that an additional antigen tube (TB2) containing peptides is used to elicit both $CD8^+$ and $CD4^+$ T-lymphocyte responses. Like its predecessors, QFT-Plus includes an antigen tube (TB1) designed to assess IFN-γ responses from $CD4^+$ helper T-lymphocytes. QFT-Plus no longer includes the antigen TB-7.7, present in prior generations of this assay, but retains the two other antigens, ESAT-6 and CFP-10. The theoretical advantages of assessing a $CD8^+$ T-cell response include improved performance in immunocompromised states that affect $CD4^+$ T-cell responses (e.g., HIV), discrimination between LTBI and active TB, and increased activity in patients with recently acquired infection [44–46]. However, to date studies have not supported these theoretical advantages.

To perform the QFT assay, whole blood is collected directly into four collection tubes, 1 ml of blood per tube, and shaken ten times [47]. Alternatively, at least 5 ml of whole blood may be collected into a lithium-heparinized tube, inverted several times, and maintained at room temperature for up to 12 h or refrigerated for up to 48 h until transfer into the QFT-Plus collection tubes [47]. After incubation for 16–24 h at 37 °C, plasma is harvested from each tube and the concentration of IFN-γ is determined for each by ELISA. The QFT-Plus assay is considered positive if the difference between TB antigen tube and the Nil tube is ≥0.35 IU/ml. To control for high background IFN-γ levels in the Nil tube, the IFN-γ response to antigen must be 25% greater than the IFN-γ concentration in the Nil control. The plasma sample from the Mitogen tube serves as an IFN-γ positive control, demonstrating T-cell activity after stimulation (Fig. 9.2).

QFT-Plus has three potential outcomes: positive, negative, or indeterminate. A low response to Mitogen (<0.5 IU/ml) indicates an indeterminate result when a blood sample also has a negative response to the TB antigens. An indeterminate response may also result from a Nil tube that has a very high background IFN-γ level (>8 IU/ml).

Interpretation criteria for the QuantiFERON-TB Gold In-Tube Test (QFT-GIT)

Interpretation	Nil*	TB Response†	Mitogen Response§
Positive¶	≤8.0	≥0.35 IU/ml and ≥25% of Nil	Any
Negative***	≤8.0	<0.35 IU/ml or <25% of Nil	≥0.5
Indeterminate††	≤8.0	<0.35 IU/ml or <25% of Nil	<0.5
	>8.0	Any	Any

Source: Based on Cellestis Limited. QuantiFERON-TB Gold In-Tube [Package insert]. Available at http://www.cellestis.com/IRM/content/pdf/QuanitFeron%20US%20VerG-Jan2010%20NO%20TRIMS.pdf.

*The interferon gamma (IFN-γ) concentration in plasma from blood incbate without antigen.

†The IFN-γ concentration in plasma from blood stimulated with a single cocktail of peptides representing early secretory antigenic target-6 (ESAT-6) culture filtrate protein-10 (CFP-10), and part of TB 7.7 minus Nil.

§The IFN-γ concentration in plasma from blood stimulated with mitogen minus Nil.

¶Interpretation indicating that *Mycobacterium tuberculosis* infection is likely.

**Interpretation indicating that *M. tuberclosis* infection is not likely.

††Interpretation indicating an uncertatin likelihood of *M. tuberculosis* infection.

Interpretation criteria for the T-SPOT.TB Test (T-Spot)

Interpretation	Nil*	TB Response†	Mitogen§
Positive¶	≤10 spots	>8 spots	Any
Broderline**	≤10 spots	5, 6, or 7 spots	Any
Negative††	≤10 spots	<4 spots	
Indeterminate**	>10 spots	Any	Any
	≤10 spots	<5 spots	<20 spots

Source: Based on Oxford Immunotec Limited T-Spot. TB [Package insert]. Available at http://www.oxfordimmunotec.com/USpageInsert.

*The number of spots resulting from incubation of PBMCs in culture media without antigens.

†The greater number of spots resulting from stimulation of peripheral blood mononuclear cells (PBMCs) with two separate cocktails of peptides representing early secretory antigenic target-6 (ESAT-6) or culture filtrate proten-10 (CFP-10) minus Nil.

§The number of spot resulting from stimulation of PBMCs with mitogen without adjustment for the number of spots resulting from incubation of PBMCs without antigens.

¶Interpretation indicating that *Mycobacterium tuberculosis* infection is likely.

**Interpretation indicating an uncertain likelihood of *M. tuberclosis* infection.

††Interpretation indicating that *M. tuberculosis* infection is not likely.

Fig. 9.2 Interpretation of results for QuantiFERON-TB Gold In-Tube Test (QFT-GIT) and T-SPOT.TB Test (T-Spot). Reproduced from Centers for Disease Control and Prevention. Updated Guidelines for Using Interferon Gamma Release Assays to Detect Mycobacterium tuberculosis Infection—United States, 2010. MMWR 2010;59(No. RR-5): p. 16

4.2.2 T-SPOT.TB Procedure

For the T-SPOT assay, whole blood (minimum 2 ml) is drawn into either lithium heparin or Vacutainer CPT tube, inverted 8–10 times, and stored at room temperature [48]. Processing must occur within 8 h unless T-cell Xtend additive is used, in which case the tubes should be kept between 10 °C and 25 °C and processed within 32 h. After centrifugation, PBMCs are extracted and washed, enumerated, and added to microtiter wells at 2.5×10^5 PBMCs per well. Although the T-SPOT step of WBC separation is technically more complex to process than QFT, it ensures a fixed number of WBCs in the assay, which may be important for immunosuppressed patients [13].

The T-SPOT assay uses four wells per patient: the negative-control (Nil) well that measures background IFN-γ-producing T-cells (spot-forming cells [SFC]), the two antigen wells (Panels A and B) separately containing ESAT-6 and CFP-10 that measure *M. tuberculosis*-specific SFC, and the positive control (Mitogen) well that measures nonspecific SFC. PBMCs and panels/controls are added to each well and placed in an incubator for 16–20 h. Wells are washed away and IFN-γ is detected via a sandwich capture technique by conjugation with secondary antibodies revealing "spots," which are counted to determine the resulting interpretation.

T-SPOT results are interpreted by subtracting the spot count in the Nil well from that in Panels A and B. T-SPOT.TB testing can result in positive, negative, or invalid (equivalent to an indeterminate QFT result) (Fig. 9.2). However, different from the rest of the world, in the USA there is a fourth category, termed "borderline." Outside of the USA, the T-SPOT is positive if Panel A-Nil and/or Panel B-Nil ≥6 spots and negative if both Panel A-Nil and Panel B-Nil ≤5 spots [48]. A Nil spot count in excess of 10 spots is considered invalid. When the mitogen well spot count is <20 spots, the result should be considered invalid unless either Panel A or Panel B are positive or borderline in which case the result is valid. In the USA, T-SPOT is considered positive when Panel A-Nil and/or Panel B-Nil ≥8 spots and negative if both Panel A-Nil and Panel B-Nil ≤4 spots; if the higher of Panel A-Nil or Panel B-Nil is 5, 6, or 7 spots, then the result is "borderline." Borderline results should be interpreted in conjunction with the patient's pretest probability of infection with *M. tuberculosis* [49].

4.2.3 Indeterminate/Invalid Results

Optimally, an indeterminate result indicates that knowledge of *M. tuberculosis* infection cannot be obtained from the IGRA due to either a low lymphocyte count or low lymphocyte response to mitogen. A study from New York City reviewed the frequency of QFT indeterminate results from public health clinics and found that 2% were indeterminate, approximately equally divided between high Nil and low Mitogen results [50]. Indeterminate frequencies of 4% have been reported in QFT results from outpatients [51] and QFT and T-SPOT results in children [52]. Very high frequencies of indeterminate results have been reported from the testing of

inpatients [53, 54]. An increased frequency of IGRA indeterminate/invalid results has been associated with young age [50, 55], Asian ethnicity [50, 51], HIV infection [55, 56], non-HIV-related immunocompromise [52], and anemia [51]. Delays in incubating the tubes are associated with indeterminate results [21]. Limited data suggests that indeterminate/invalid results are more common for QFT than T-SPOT when the CD4 count is <200 cells/μl [13].

In the case of indeterminate/invalid results, IGRA manufacturers recommend recollection of blood and retesting with the same assay; approximately two-third of repeat tests will give an actionable result (i.e., positive or negative) [50].

4.2.4 Sources of Variation/Error

Like any laboratory test, IGRAs are subject to variability at every step of the process. These sources have been categorized as preanalytical, analytical, manufacturing, and immunological [57]. Although most evaluations have been with QFT, these sources of variation likely apply to T-SPOT.

Significant preanalytical causes of variability include inadequate blood volume, failure to invert tubes after collecting blood, overly vigorous shaking of tubes, and delays in the processing of tubes. A systematic review identified blood volume inoculated into IGRA tubes and delay in processing as key sources of variability [58].

Analytical sources include variation in laboratory techniques and imprecision in measurements. One study that retested the same blood samples with QFT-GIT found that the within-subject variability in IFN-γ response on retesting was 0.60 IU/ml for all persons, and 0.24 IU/ml in individuals whose initial TB response was near the QFT cut-off (0.25–0.80 IU/ml) [59]. This "normal" variation resulted in conversion and reversion rates of 9% and 7%, respectively. A systematic review on the reproducibility of IGRAs, found that the estimated range of variability of IFN-γ response in QuantiFERON under identical conditions was ±0.47 IU/ml (coefficient of variation, 13%) overall, and ±0.26 IU/ml (30%) for individuals with an initial IFN-γ response 0.25–0.80 IU/ml, near the QFT cut-point [60]. Due to this variation in QFT result, a change in LTBI diagnosis was not uncommon: 26% of samples converted to positive if the baseline result ranged between 0.25 and 0.34 IU/ml, and 18% of samples reverted to negative if baseline IFN-γ results were between 0.35 and 0.8 IU/ml.

Immunological sources of variation include immune boosting in the setting of recent TST. Boosting of an IGRA result has been reported in the setting of recent TST administration. Dorman et al performed repeat IGRA testing (both QFT-GIT and T-SPOT) 7–21 days after IGRA and TST testing in persons with IGRAs that were negative at baseline [20]. A boosted response (i.e., conversion to a positive IGRA) was observed in 9.1% of participants by QFT-GIT and 11.3% by T-SPOT. This phenomenon was more common if the baseline testing result was TST+/IGRA−, in agreement with prior studies [61]. IGRA boosting due to TST administration appears to start 3 or more days post-TST placement and may wane by 6 months

after the TST. ATS/IDSA/CDC guidelines recommend that when dual testing (i.e., TST and IGRA) is considered that the IGRA be collected either concurrently with or prior to TST placement [13].

4.3 Accuracy

As previously noted, the lack of direct tests to identify *M. tuberculosis* infection has led to most studies of LTBI test accuracy using surrogate outcomes: estimating sensitivity in patients with current or prior TB and specificity in persons at low risk for *M. tuberculosis* infection.

Across many studies, IGRA sensitivity was equal to (QFT 81–86%) or superior to (T-SPOT 90–95%) the sensitivity of the TST (71–82%) when the outcome was microbiologically confirmed or clinical TB [13]. False-negative IGRA results are associated with advanced age, HIV infection, non-HIV-related immunocompromise, low lymphocyte count, and extrapulmonary forms of TB including CNS, pleural, and bone and joint TB [60].

Among persons who have not received vaccination with BCG, the specificity of IGRAs and TST is similar (>95%) [13]. In BCG vaccinated individuals, IGRAs offer improved specificity over the TST: >95 vs. 60% [13, 21]. Despite findings of similar or superior specificity with IGRAs compared to the TST, IGRAs have proved less specific in populations at low risk for infection with *M. tuberculosis*, including US health care workers [20, 62] and US-born individuals [19].

Using data from the National Health and Nutrition Examination (NHANES), a sample representative of the US population, Ghassemieh et al investigated QFT and TST agreement in more than 6000 individuals [19]. Using a TST response of 10 mm of induration and the manufacturer's recommended QFT-GIT cut-point, test agreement in US-born participants was 97.0% although the kappa statistic was only fair at 0.27 (95% CI, 0.18–0.36). Among US-born participants, 0.6% were positive by both tests, 0.8% were TST-positive only, and 2.2% were IGRA-positive only. Among non-US-born participants, test agreement was 81.6% with kappa statistic 0.38 (95% CI, 0.33–0.44). Test results in non-US-born participants were: 9.1% positive by both tests, 11.2% TST-positive only, and 7.2% IGRA-positive only.

Dorman and colleagues performed a longitudinal study of QFT-GIT, T-SPOT. TB, and TST in over 2400 US healthcare workers [20]. All participants were considered at low risk for infection with *M. tuberculosis* and follow-up visits occurred at 6, 12, and 18 months after enrollment. A positive test at study enrollment was present in 1.8% by TST, 3.8% by QFT-GIT, and 5.0% by T-SPOT. Among participants with negative test results at enrollment, conversion to a positive test result (likely a false-positive result) occurred in 0.9% by TST, 6.1% by QFT-GIT, and 8.3% by T-SPOT. Test conversions were six to nine times more frequent with IGRAs than TST. For both IGRAs, the likelihood of a conversion or reversion increased if the baseline quantitative value was closer to the test cut-point. Among participants

who were positive at enrollment, reversions occurred in 57% with QFT and 64% with T-SPOT; reversions were very common for baseline QFT values <0.7 IU/ml and T-SPOT values ≤10 SFCs. The Ghassemieh and Dorman studies point to compromised IGRA specificity in populations at low risk for LTBI.

A study from a moderate TB-burden setting, rural China, where LTBI risk is high, enrolled over 21,000 participants to undergo testing with the TST and QFT [63]. Age- and sex-standardized rates of TST positivity (≥10 mm) ranged from 15 to 42%, and QFT positivity rates ranged from 13 to 20%. TST-only positive results were associated with the presence of a BCG scar. Out of this cohort, 7505 participants with a positive TST and/or QFT who were not treated for LTBI were followed for 2 years [64]. The TB incidence rate was 0.87 per 100 person-years among participants who tested positive with QFT, 0.50 per 100 person-years for those who tested positive with TST, and 0.82 per 100 person-years for those who tested positive with both tests.

Several points are worth emphasizing based on the above studies. These results support the preferential use of IGRAs in BCG vaccinated individuals. However, QFT and T-SPOT specificity seem to be lower than the TST in low-risk populations, related in part to the use of a single cut-point for IGRAs. Second, people at low risk for LTBI should not be tested. For example, using an IGRA with sensitivity of 86% and specificity of 95% to test 1000 US-born individuals (LTBI prevalence of 2.7%) [19] would result in 23 true-positive persons and 49 false positives: 68% of the positive test results would be false positive. Whereas using the same test in foreign-born residents of the USA (15.9% LTBI prevalence) would identify 137 true-positives and 42 false-positive results: 23% of the positive results would be false positive. Third, IGRA values close to the cut-point (0.35 IU/ml for QFT and 6 SFCs for T-SPOT) may be read as positive due to laboratory variability. It is important to examine the magnitude of the test result and the pretest probability of LTBI in interpreting an IGRA result.

5 Comparisons of TST, QFT, and T-SPOT.TB

The majority of studies on LTBI test accuracy have used surrogate outcomes. However, at least one large study from a low TB-burden setting has evaluated the performance of the three commercially available tests in predicting progression to active TB [65]. This is significant as the goal of LTBI screening and treatment is to prevent TB disease. UK PREDICT TB was an English study that enrolled persons at high risk for LTBI (i.e., close contacts to a patient with infectious TB, recent immigrants from high TB-burden countries) to undergo QFT-GIT, T-SPOT, and TST and followed enrollees until study completion (median 2.9 years) [65]. Several thresholds were evaluated for determining a positive TST including 5 mm, 10 mm, and a novel strategy of 5 mm for BCG-naïve and 15 mm for BCG vaccinated participants (TST-15). Among 6380 participants, 83% of whom were born outside the UK, 1.2% developed TB. In these patients, QFT was positive in 61%, T-SPOT positive in 68%, TST (5 mm) positive in 83%, and TST-15 positive in 68%. The positive

predictive values for the tests were: T-SPOT 4.2%, QFT-GIT 3.3%, and TST-15 3.5%. A positive T-SPOT result was a significantly better predictor of progression to TB than the other tests. Negative predictive values were similar across tests and TST thresholds ranging from 99.4 to 99.6%.

5.1 Alternative Cut-Points

Unlike the TST, for which risk-based thresholds are applied in determining a positive result, IGRAs were approved with a single cut-point value (excepting T-SPOT in the USA which includes a borderline category). As discussed in *Sources of Variation* and demonstrated by the Dorman study [20], patients with IGRA results close to the cut-point may experience conversions and/or reversions during serial testing; most of these patients will not have infection with *M. tuberculosis*. Studies have evaluated whether alternative IGRA cut-points could improve test accuracy in diagnosing *M. tuberculosis* infection.

Higher quantitative IGRA results are associated with an increased risk of progression to active TB [66–69]. A systematic review and meta-regression analysis evaluated whether antigen-nil IFN-γ levels correlate with risk of progression to TB [70]. Based on 34 included studies, the investigators found that higher levels of IFN-γ were associated with increased risk of progression to TB in a dose–response relationship. Whether these findings are reflective of a biologic mechanism or increasing specificity with higher IFN-γ levels is not known.

A study from South Africa, a very high-burden setting, found that QFT values >0.7 IU/ml were strongly associated with progression to TB whereas "positive" QFT results below this range (i.e., 0.35–0.7 IU/ml) did not have an increased risk of TB compared to QFT-negative individuals [71]. However, given the small number of participants who progressed to TB, this study may have been underpowered and should not be applied to low-burden settings.

In 2010, Sweden introduced a borderline range for QFT results between 0.2 and 0.99, with a recommendation to repeat the test if initial values fall within this range [72]. Swedish investigators evaluated over 40,000 QFT-GIT test results, from which 9% were within the borderline range. On retesting of these borderline results, 54% were <0.35 IU/ml (i.e., negative), 27% remained borderline positive (0.35–0.99 IU/ml), and 17% had a value >0.99 IU/ml. No patients with an initial borderline result developed TB within 3–24 months. Similar findings have been reported from South Korea [73].

However, other studies from low-burden settings have called into question the use of a borderline category. Gupta and colleagues evaluated data from a UK cohort of 9610 TB contacts and recent immigrants to identify QFT, T-SPOT, and TST cut-points that would improve on test specificity while maintaining adequate sensitivity [67]. Although TB incidence increased with the magnitude of test responses, loss of sensitivity with higher thresholds supported keeping the current QFT and T-SPOT cut-points. A study of Portuguese healthcare workers suggested restricting the use of borderline categories to low TB risk populations only [74].

It remains unclear whether a different threshold for IGRA positivity would be of use in clinical practice to improve specificity without significantly decreasing sensitivity. The authors follow the US guidelines to test for LTBI only when risk factors are present [13]. When patients with a low pretest probability for *M. tuberculosis* infection are tested, we recommend evaluating the magnitude of the test response. For low-risk patients, if the first test result is weakly positive, we perform a second test and offer treatment only if both tests are positive.

6 Test Application in Special Situations and Populations

6.1 Marker of Treatment Response

LTBI treatment has no consistent effect on IGRA values [75–79]. This means that measuring IFN-γ levels pre- and post-treatment is not useful as a measure of treatment response.

6.2 Pediatrics

The 2017, American Thoracic Society/Infectious Diseases Society of America/CDC guidelines on TB diagnosis recommend IGRAs as preferable or equivalent to the TST in children aged 5 years and older [13]. However, for children <5 years of age, the guidelines recommend the TST over IGRAs. This was based on limited direct evidence at the time of publication that suggested that the TST has greater sensitivity but lower specificity than IGRAs in young children. Because of the high risk of progression to active TB in children <5 years, the guidelines prioritized sensitivity over specificity in this population. Differing slightly from these recommendations, the American Academy of Pediatrics preferentially recommends the TST in children <2 years of age [80], based on greater experience and understanding of its performance in this young population compared to IGRAs. In addition, phlebotomy is more difficult in young children and insufficient blood volumes may be an added reason for performing TST in this population.

Using TBESC data, Ahmed and colleagues evaluated the performance of QFT-GIT, T-SPOT, and TST in more than 3500 children <15 years of age [81]. Four children developed active TB. The negative predictive values for TST, QFT-GIT, and T-SPOT were 99.9 (95% CI: 99.7–100), 100 (95% CI: 99.8–100), and 99.9 (95% CI: 99.8–100), respectively. Of 533 children with TST-positive/IGRA-negative results who were not treated for LTBI (including 54 children <2 years old), none developed active TB.

These findings suggest that IGRAs are likely accurate in testing for LTBI in children of all ages. The TST remains an acceptable alternative, although its lower specificity will result in a higher frequency of positive results.

6.3 Pregnancy

A systematic review from 2016 identified three studies of LTBI performance in pregnant women from low-burden settings, with concordance between TST and IGRAs of 77, 88, and 91%. The authors concluded that in low-burden settings, test performance was not impacted by pregnancy [82]. Differing from low-burden settings, this systematic review identified one study from a high-burden setting (India) in which all participants were HIV-negative and found that QFT was positive twice as often as the TST. In addition, they observed that the positivity rate for both QFT and TST increased in the postpartum period compared to antepartum [83]. Among pregnant women with HIV in high TB-burden settings, IGRAs were found to have about twice the positivity rate of the TST, and the positivity rate was higher 3 months postpartum compared to antepartum [84, 85]. Collectively these studies suggest that LTBI testing should be delayed during pregnancy unless there is a strong indication (e.g., recent contact to a person with infectious TB, or newly diagnosed HIV). In addition, IGRAs may be more sensitive tests than the TST in peripartum women.

6.4 Serial Testing

As discussed above, serial testing in low-risk populations (e.g., health care workers) is discouraged by the US CDC [86]. When testing is performed, IGRAs may result in a much higher proportion of conversions than the TST (6–8 times higher [20]). New IGRA conversions should be carefully evaluated for the magnitude of the result (IU/ml or SFCs) and repeat testing should be considered when the conversion value is near the cut-point of the test.

6.5 People with HIV

The sensitivity of IGRAs and the TST are decreased in people with HIV. After reviewing the literature, the US guidelines found that the sensitivity of both IGRAs for detecting LTBI in people living with HIV is between 65 and 100%, compared to the estimated sensitivity of the TST at 43% [13]. Although this data suggests that IGRAs are at least as sensitive as TST in people living with HIV, the guideline committee decided that there was insufficient data to recommend IGRAs over TST.

A TBESC study used Bayesian latent class analysis, a statistical technique that provides an understanding of test characteristics when no gold standard is available, to estimate the accuracy of the three commercially available tests for the diagnosis of LTBI in US-born people living with HIV [87]. The investigators found that T-SPOT had a significantly higher positive predictive value (90.0%) than QFT (50.7%) and TST (45.4%) and similar negative predictive values across the three tests. The estimated sensitivity was higher for QFT (72.2%) than T-SPOT (51.9%) or TST (54.2%) [87].

Differing from these results, a recent systematic review of TB risk after a positive LTBI test included 9 cohorts of people living with HIV from low TB-burden settings [88]. The incidence of TB was 16.9 per 1000 person-years with a positive IGRA result and 27.1 per 1000 person-years with a positive TST result of ≥5 mm. Based on conflicting findings and a small evidence base, it seems appropriate that the US guidelines do not endorse one test over the other in the setting of HIV infection.

6.6 Other Immunocompromise

The performance of LTBI tests likely varies between immunocompromising conditions give differences in etiologies and immune dysfunction. For example, in one study 41% of pre-liver transplant patients had indeterminate QuantiFERON results compared to 12% of non-liver transplant patients [89]. A recent systematic review of LTBI diagnosis in transplant candidates found that IGRAs were more sensitive and specific than the TST with regard to the diagnosis of LTBI in transplant candidates, although all tests had sub-optimal performance: sensitivity was 46%, 58%, and 55% for the TST, QFT, and T-SPOT, respectively [90]. Specificity of TST, QFT, and T-SPOT were 86%, 89%, and 92%, respectively. Likewise, among patients dependent upon hemodialysis, IGRAs had superior accuracy to the TST [91].

There is no consensus among the various transplant societies as to the preferred LTBI diagnostic strategy for transplant candidates [16]. Among the different transplant organizations, recommended strategies include preferential use of an IGRA, the TST, 2-step TSTs (to take advantage of boosting), either TST or IGRA, and both tests (if the first is negative). The transplant program at the authors' institution preferentially uses an IGRA for LTBI testing [92]. In the setting of LTBI risk factors (e.g., birth in a moderate- or high-burden country), a second test may be performed if the first is negative and treatment offered if the second test is positive. As most solid organ transplant recipients who develop TB will have had a negative TST and/

or IGRAs on pretransplant testing [93], when there is a high pretest probability for LTBI or concern over false-negative test results, some experts offer LTBI treatment regardless of TST and IGRA results [92].

6.7 Society Recommendations

The US ATS/IDSA/CDC guidelines, published in 2017, make the following recommendations (Fig. 9.3):

- A strong recommendation to perform an IGRA rather than a TST in individuals ≥5 years of age who are likely to be infected with *M. tuberculosis*, have a low or intermediate risk of disease progression, and either have a history of BCG vaccination or are unlikely to return to have their TST read.
- A conditional recommendation is to perform an IGRA rather than a TST in all other individuals ≥5 years of age who are likely to be infected with *M. tuberculosis* and who have a low or intermediate risk of disease progression.
- Based on a lack of data, no test preference recommendation for individuals ≥5 years of age who are likely to be infected with *M. tuberculosis* and who have a high risk of progression to disease.
- A recommendation to NOT test persons at low risk for *M. tuberculosis* infection and disease progression. If diagnostic testing for LTBI is performed in individuals who are unlikely to be infected with *M. tuberculosis,* an IGRA instead of a TST is recommended for persons ≥5 years. If this test is positive, then a second test is recommended. The confirmatory test may be either an IGRA or a TST and the patient is considered infected only if both tests are positive.
- TST rather than an IGRA is recommended for healthy children <5 years of age.

Guidance published by the European Centre for Disease Prevention and Control (ECDC) in 2018 recommends the preferential use of IGRAs in people with a history of BCG vaccination, migrant populations, and hard-to-reach populations (the latter two groups based on the need for only a single visit) [94]. The TST is recommended for children <5 years and a combination of TST and IGRA in immunocompromised patients to maximize sensitivity. For other tested populations, no general recommendation is made as to which test is preferred but rather it should be based on country-specific circumstances, operational issues, and patient considerations.

Group	Testing Strategy	Considerations
Likely to be Infected **High** Risk of Progression (TST ≥ 5mM)	**Adults** **Acceptable:** IGRA OR TST Consider dual testing where a positive result from either result would be considered **positive** **Children ≤ 5years of age** **Preferred:** TST **Acceptable:** IGRA OR TST Conside dual testing where a positive result from either would be considered **positive**[1]	Prevalence of BCG vaccination Expertise of staff and/or laboratory Test availability Patient perceptions Staff perceptions Programmatic concerns
Likely to be Infected **Low** to **Intermediate** Risk of Progression (TST ≥ 10mM)	**Preferred:** IGRA where available **Acceptable:** IGRA or TST	
Unlikely to be Infected (TST ≥ 15mM)	**Testing for LTBI is not recommended** **If necessary:** **Preferred:** IGRA where available. **Acceptable:** Either IDRA OR TST **For serial testing:** **Acceptable:** Either IGRA OR TST Consider repeat or dual testing where a negative result from either would be considered **negative**[2]	

Fig. 9.3 Summary of recommendations for testing for latent tuberculosis infection (LTBI). (1) Performing a second diagnostic test when the initial test is negative is a strategy to increase sensitivity. This may reduce specificity, but the panel decided that this is an acceptable trade-off in situations in which the consequences of missing LTBI (i.e., not treating individuals who may benefit from therapy) exceed the consequences of inappropriate therapy (i.e., hepatotoxicity). (2) Performing a confirmatory test following an initial positive result is based upon both the evidence that false-positive results are common among individuals who are unlikely to be infected with *Mycobacterium tuberculosis* and the committee's presumption that performing a second test on those patients whose initial test was positive will help identify initial false-positive results. Abbreviations: *IGRA* interferon-γ release assay, *LTBI* latent tuberculosis infection, *TST* tuberculin skin test. Reproduced with permission [13]

7 Future Directions of LTBI Testing

There is an urgent need for new LTBI diagnostics that address the limitations of the current testing platforms. Performance characteristics of critical importance for future LTBI diagnostics include the capability to:

- Discriminate between subclinical disease, LTBI, and past infection.
- Identify those at elevated risk of progression to TB disease.
- Assess response to therapy.

Other needed characteristics include ease of use, affordability, and ability to provide rapid, easily interpretable results at the point of care.

While no new diagnostic platform is currently poised to replace the current TST and IGRA tests for the diagnosis of LTBI, a wide variety of technologies are at different phases of investigation, development, or clinical use. Many aim to improve on current immunodiagnostics while others use entirely new diagnostic strategies.

7.1 Novel Skin-Based Tests

A number of novel skin-based tests for LTBI diagnosis have been designed to elicit a more *M. tuberculosis*-specific immune response than the conventional TST while remaining low cost and low technology. The C-Tb (Serum Institute of India, Pune, India), Diaskintest (Generium, Moscow, Russia), and C-TST (Anhui Zhifei Longcom, Hefei, China) all use the same recombinant ESAT-6 and CFP-10 proteins used in IGRAs [95–97], whereas the DPPD test (Host Directed Therapeutics Bio Corp, Seattle, WA, USA) uses a different recombinant protein specific to *M. tuberculosis* [98].

A recent systematic review and meta-analysis evaluated the evidence for the diagnostic performance of these novel skin-based tests compared to the standard TST or IGRAs [99]. All four tests were found to have similar performance to the TST and IGRAs. While test specificity can be as high as IGRAs [95, 100], overall, variations between the performance of the novel skin-based tests and TST or IGRA were small. All four novel tests were judged to likely have similar value as the conventional tests in identifying people most at risk of developing active TB.

7.2 Novel Cytokine-Based Assays

Strategies to improve the performance of current IGRAs have primarily focused on the use of different immunogenic antigens or the detection of alterative, non-IFN-γ cytokines.

7.2.1 Use of New Antigens

One novel IGRA test that uses a combination of conventional and novel antigens is the LIOFeron TB/LTBI (Lionex GmbH, Braunschweig, Germany), which was introduced in 2019 [101]. The assay is similar to older versions of the QFT assay, where the first antigen tube has ESAT-6 and CFP-10, as well as TB-7.7. However, the test differs in that it has a second antigen tube that contains a recombinant version of *M. tuberculosis* alanine dehydrogenase (Ala-DH). The Ala-DH is not found in BCG (similar to the other antigens) and is known to have a number of epitopes for $CD8^+$ T-cells [102]. While one peer-reviewed study suggested that the LIOFeron test may have a similar performance to QFT [101], more controlled studies are needed.

Many other novel antigens are in the preclinical stages of evaluation [103–106]. To address the need for an IGRA that does not contain ESAT-6, a fundamental component of many experimental TB vaccine candidates which render conventional IGRAs nonspecific following vaccination, Ruwald and colleagues developed an ESAT-6 free IGRA that uses the antigens EspC, EspF, and Rv2348c in combination with CFP-10 [107]. On initial assessment, the ESAT-6-free IGRA had a similar diagnostic performance to QFT. In another study, combining the same novel antigens with a standard QFT assay yielded higher sensitivity, particularly among patients with impaired immune systems, without loss of specificity [108].

7.2.2 Use of Alternative Cytokines

A wide range of cytokine responses beyond IFN-γ has been investigated for their potential to improve the diagnosis of LTBI. A recent systematic review of studies of ESAT-6/CFP-10 cytokine responses for the differentiation of LTBI from active TB identified 100 different cytokines under investigation, with the most frequently studied being IL-2, TNF-α, IP-10, IL-10, and IL-13, in addition to IFN-γ [109]. One of the best studied non-IFN-γ cytokines is IP-10, an IFN-γ-induced protein that is expressed at 100-times higher levels than IFN-γ [110]. Multiple studies have suggested that IP-10 release assays perform as well as IGRAs [110–112]. Moreover, IP-10 release assays have some advantages, including robust performance even when used on dry blood spots, which are convenient for collection, transport, and storage [113, 114].

In recent years, investigations into the use of alternative cytokine detection for diagnosing LTBI have begun to move away from profiling individual cytokine responses and toward identifying complex, multi-cytokine signatures [115]. In one recent study, researchers isolated PBMCs from the blood of 92 subjects including those with active TB, LTBI, and healthy controls, and cytokine production in response to PPD stimulation was measured by a multiplex immunoassay system [116]. Analyzing the results with a machine learning algorithm, the researchers were able to identify a two-cytokine combination that distinguished LTBI from active TB better than any individual cytokine (84% sensitivity, 89% specificity). In another similar study, researchers isolated PBMCs from 65 subjects with various

known TB risk factors including LTBI and assessed cytokine response to a variety of *M. tuberculosis*-associated antigens using a 13-target multiplex immunoassay [117]. Using a machine learning algorithm, the investigators identified multi-cytokine signatures that could predict LTBI diagnosis and relative risk designation with >80% accuracy.

7.3 Serology

Serologic tests based on antibodies against *M. tuberculosis* have the advantages of being simple, inexpensive, and amenable to point-of-care diagnostics. Historically, though, such assays have not been oriented toward LTBI diagnosis and their poor sensitivity and specificity have limited their utility [118]. However, a growing understanding of the association between specific immunogenic antigens and different phases of infection and disease [119], including LTBI [120–122], suggests that antibody-based testing may play a role in future diagnostics for LTBI.

7.4 Transcriptomics

RNA transcriptomics, the study of host gene expression by whole blood RNA sequencing, has shown substantial potential for advancing the diagnosis of LTBI. Numerous studies have suggested that transcriptomic signatures may help identify individuals at elevated risk of active TB and may help differentiate between different phases of infection and disease [123–128]. However, it is an imperfect predictor of risk—as underscored by the results of the large CORTIS trial in 2021, which sought to assess the use of a transcriptomic signature (RISK11) to identify individuals at high risk of TB and prevent disease through targeted use of TB preventative therapy [129]. RISK11 had only very modest predictive performance, and the targeted TB preventative therapy did not reduce progression to active TB over the follow-up period. While transcriptomic signatures may play a role in at least short-term prediction of TB risk [130], it is still unclear how to best interpret positive tests, how much absolute risk they predict, and whether they should be combined with other predictive factors [131].

7.5 Other Novel Diagnostic Strategies

While beyond the scope of this chapter, there are many other potential approaches to improving the diagnosis of LTBI that are under investigation including proteomic and metabolomic profiling, the characterization of $CD4^+$ T-cell activation markers, and the use of combinatorial algorithms that bring together clinical, immunologic, and other factors.

8 Conclusion

The current approach to the diagnosis of LTBI to prevent TB relies on detecting evidence of cell-mediated immunity to *M. tuberculosis* antigens. If testing is positive and there are no clinical signs or symptoms of TB disease, the patient is typically considered to have LTBI. There is no current test capable of directly determining the presence of infection with *M. tuberculosis*.

The two types of LTBI diagnostics widely available are the TST and IGRAs. The advantages of the TST include lower cost, ease of administration, no lab requirement, and adjustable interpretation cut-off per individual LTBI risks. The advantages of IGRAs include requiring a single visit, no cross-reactivity with BCG vaccination or most NTM, and likely modestly improved sensitivity (particularly with immunocompromised individuals). Of note, the TST and IGRA have an only poor-to-fair concordance, like due in part to greater cross-reactivity issues with the TST.

The shortcomings of both the TST and IGRAs are significant. These include the fact that they cannot discriminate asymptomatic infection from past infection, identify individuals at elevated risk of TB disease, or be used to assess response to preventative therapy. For these reasons, a variety of novel technologies are in different phases of investigation, development, or clinical use that may help address these issues. These include novel skin-based tests, enhanced cytokine-release assays, and testing for RNA transcriptomic signatures.

References

1. Pai M, Sotgiu G. Diagnostics for latent TB infection: incremental, not transformative progress. Eur Respir J. 2016;47(3):704–6. https://doi.org/10.1183/13993003.01910-2015.
2. Churchyard G, Kim P, Shah NS, Rustomjee R, Gandhi N, Mathema B, et al. What we know about tuberculosis transmission: an overview. J Infect Dis. 2017;216(Suppl 6):S629–S35. https://doi.org/10.1093/infdis/jix362.
3. Patterson B, Wood R. Is cough really necessary for TB transmission? Tuberculosis. 2019;117:31–5. https://doi.org/10.1016/j.tube.2019.05.003.
4. Torrelles JB, Schlesinger LS. Integrating lung physiology, immunology, and tuberculosis. Trends Microbiol. 2017;25(8):688–97. https://doi.org/10.1016/j.tim.2017.03.007.
5. Ernst JD. The immunological life cycle of tuberculosis. Nat Rev Immunol. 2012;12(8):581–91. https://doi.org/10.1038/nri3259.
6. Kornfeld H, Mancino G, Colizzi V. The role of macrophage cell death in tuberculosis. Cell Death Differ. 1999;6(1):71–8. https://doi.org/10.1038/sj.cdd.4400454.
7. Pai M, Behr MA, Dowdy D, Dheda K, Divangahi M, Boehme CC, et al. Tuberculosis. Nat Rev Dis Primers. 2016;2(1):16076. https://doi.org/10.1038/nrdp.2016.76.
8. Mack U, Migliori GB, Sester M, Rieder HL, Ehlers S, Goletti D, et al. LTBI: latent tuberculosis infection or lasting immune responses to M. tuberculosis? A TBNET consensus statement. Eur Respir J. 2009;33(5):956–73. https://doi.org/10.1183/09031936.00120908.
9. Esmail H, Barry CE, Young DB, Wilkinson RJ. The ongoing challenge of latent tuberculosis. Philos Trans R Soc Lond B Biol Sci. 2014;369(1645):20130437. https://doi.org/10.1098/rstb.2013.0437.

10. Ramakrishnan L. Revisiting the role of the granuloma in tuberculosis. Nat Rev Immunol. 2012;12(5):352–66. https://doi.org/10.1038/nri3211.
11. Maher D. Chapter 13. The natural history of *Mycobacterium tuberculosis* infection in adults. In: Simon H, Schaaf AZ, editors. Tuberculosis: a comprehensive clinical reference. Philadelphia: Saunders; 2009. p. 129–32.
12. Orme IM, Robinson RT, Cooper AM. The balance between protective and pathogenic immune responses in the TB-infected lung. Nat Immunol. 2015;16(1):57–63. https://doi.org/10.1038/ni.3048.
13. Lewinsohn DM, Leonard MK, LoBue PA, Cohn DL, Daley CL, Desmond E, et al. Official American Thoracic Society/Infectious Diseases Society of America/Centers for Disease Control and Prevention Clinical Practice Guidelines: diagnosis of tuberculosis in adults and children. Clin Infect Dis. 2017;64(2):e1–e33. https://doi.org/10.1093/cid/ciw694.
14. Drain PK, Bajema KL, Dowdy D, Dheda K, Naidoo K, Schumacher SG, et al. Incipient and subclinical tuberculosis: a clinical review of early stages and progression of infection. Clin Microbiol Rev. 2018;31(4):e00021–18. https://doi.org/10.1128/cmr.00021-18.
15. Actor JK. Introductory immunology. In: Basic concepts for interdisciplinary applications. 2nd ed. Atlanta, GA: Elsevier; 2019.
16. Munoz L, Santin M. Prevention and management of tuberculosis in transplant recipients: from guidelines to clinical practice. Transplantation. 2016;100(9):1840–52. https://doi.org/10.1097/TP.0000000000001224.
17. Pai M, Behr M. Latent *Mycobacterium tuberculosis* infection and interferon-gamma release assays. Microbiol Spectr. 2016;4(5) https://doi.org/10.1128/microbiolspec.
18. Arend SM, Meijgaarden KEV, Boer KD, Palou ECD, Soolingen DV, Ottenhoff THM, et al. Tuberculin skin testing and in vitro T cell responses to ESAT-6 and culture filtrate protein 10 after infection with *Mycobacterium marinum* or *M. kansasii*. J Infect Dis. 2002;186(12):1797–807. https://doi.org/10.1086/345760.
19. Ghassemieh BJ, Attia EF, Koelle DM, Mancuso JD, Narita M, Horne DJ. Latent tuberculosis infection test agreement in the National Health and Nutrition Examination Survey. Am J Respir Crit Care Med. 2016;194(4):493–500. https://doi.org/10.1164/rccm.201508-1560OC.
20. Dorman SE, Belknap R, Graviss EA, Reves R, Schluger N, Weinfurter P, et al. Interferon-gamma release assays and tuberculin skin testing for diagnosis of latent tuberculosis infection in healthcare workers in the United States. Am J Respir Crit Care Med. 2014;189(1):77–87. https://doi.org/10.1164/rccm.201302-0365OC.
21. Pai M, Denkinger CM, Kik SV, Rangaka MX, Zwerling A, Oxlade O, et al. Gamma interferon release assays for detection of *Mycobacterium tuberculosis* infection. Clin Microbiol Rev. 2014;27(1):3–20. https://doi.org/10.1128/CMR.00034-13.
22. Iseman MD. A clinician's guide to tuberculosis. Philadelphia: Lippincott Williams & Wilkins; 2000.
23. Bates JH. Tuberculosis. 2nd ed. Philadelphia: Lippincott Williams & Wilkins; 2004.
24. Yang H, Kruh-Garcia NA, Dobos KM. Purified protein derivatives of tuberculin—past, present, and future. FEMS Immunol Med Microbiol. 2012;66(3):273–80. https://doi.org/10.1111/j.1574-695X.2012.01002.x.
25. Gillenwater KA, Sapp SC, Pearce K, Siberry GK. Increase in tuberculin skin test converters among health care workers after a change from Tubersol to Aplisol. Am J Infect Control. 2006;34(10):651–4. https://doi.org/10.1016/j.ajic.2006.05.288.
26. Diagnostic Standards and Classification of Tuberculosis in Adults and Children. This official statement of the American Thoracic Society and the Centers for Disease Control and Prevention was adopted by the ATS Board of Directors, July 1999. This statement was endorsed by the Council of the Infectious Disease Society of America, September 1999. Am J Respir Crit Care Med. 2000;161(4 Pt 1):1376–95. https://doi.org/10.1164/ajrccm.161.4.16141.
27. Huebner RE, Schein MF, Bass JB Jr. The tuberculin skin test. Clin Infect Dis. 1993;17(6):968–75. https://doi.org/10.1093/clinids/17.6.968.

28. Menzies D. Interpretation of repeated tuberculin tests. Boosting, conversion, and reversion. Am J Respir Crit Care Med. 1999;159(1):15–21. https://doi.org/10.1164/ajrccm.159.1.9801120.
29. Targeted tuberculin testing and treatment of latent tuberculosis infection. Statement of the ATS and the CDC. Am J Respir Crit Care Med. 2000;161:S221–47.
30. Youssef E, Wooltorton E. Serious allergic reactions following tuberculin skin tests. CMAJ. 2005;173(1):34. https://doi.org/10.1503/cmaj.050710.
31. Bunnet D, Kerleguer A, Kim P, Pean P, Phuong V, Heng N, et al. Necrotic tuberculin skin (Mantoux) test reaction: a case report and an estimation of frequency. Chest. 2015;148(1):e1–4. https://doi.org/10.1378/chest.14-2463.
32. Froeschle JE, Ruben FL, Bloh AM. Immediate hypersensitivity reactions after use of tuberculin skin testing. Clin Infect Dis. 2002;34(1):E12–3. https://doi.org/10.1086/324587.
33. Tager IB, Kalaidjian R, Baldini L, Rocklin RE. Variability in the intradermal and in vitro lymphocyte responses to PPD in patients receiving isoniazid chemoprophylaxis. Am Rev Respir Dis. 1985;131(2):214–20. https://doi.org/10.1164/arrd.1985.131.2.214.
34. Schatz M, Patterson R, Kloner R, Falk J. The prevalence of tuberculosis and positive tuberculin skin tests in a steroid-treated asthmatic population. Ann Intern Med. 1976;84(3):261–5. https://doi.org/10.7326/0003-4819-84-3-261.
35. Belard E, Semb S, Ruhwald M, Werlinrud AM, Soborg B, Jensen FK, et al. Prednisolone treatment affects the performance of the QuantiFERON gold in-tube test and the tuberculin skin test in patients with autoimmune disorders screened for latent tuberculosis infection. Inflamm Bowel Dis. 2011;17(11):2340–9. https://doi.org/10.1002/ibd.21605.
36. Bovornkitti S, Kangsadal P, Sathirapat P, Oonsombatti P. Reversion and reconversion rate of tuberculin skin reactions in correction with the use of prednisone. Dis Chest. 1960;38:51–5. https://doi.org/10.1378/chest.38.1.51.
37. Agarwal S, Das SK, Agarwal GG, Srivastava R. Steroids decrease prevalence of positive tuberculin skin test in rheumatoid arthritis: implications on Anti-TNF therapies. Interdiscip Perspect Infect Dis. 2014;2014:430134. https://doi.org/10.1155/2014/430134.
38. Stout JE, Wu Y, Ho CS, Pettit AC, Feng PJ, Katz DJ, et al. Evaluating latent tuberculosis infection diagnostics using latent class analysis. Thorax. 2018; https://doi.org/10.1136/thoraxjnl-2018-211715.
39. Farhat M, Greenaway C, Pai M, Menzies D. False-positive tuberculin skin tests: what is the absolute effect of BCG and non-tuberculous mycobacteria? Int J Tuberc Lung Dis. 2006;10(11):1192–204.
40. Thompson NJ, Glassroth JL, Snider DE Jr, Farer LS. The booster phenomenon in serial tuberculin testing. Am Rev Respir Dis. 1979;119(4):587–97. https://doi.org/10.1164/arrd.1979.119.4.587.
41. Wood PR, Corner LA, Plackett P. Development of a simple, rapid in vitro cellular assay for bovine tuberculosis based on the production of gamma interferon. Res Vet Sci. 1990;49(1):46–9.
42. Streeton JA, Desem N, Jones SL. Sensitivity and specificity of a gamma interferon blood test for tuberculosis infection. Int J Tuberc Lung Dis. 1998;2(6):443–50.
43. Lalvani A. Diagnosing tuberculosis infection in the 21st century: new tools to tackle an old enemy. Chest. 2007;131(6):1898–906. https://doi.org/10.1378/chest.06-2471.
44. Chiacchio T, Petruccioli E, Vanini V, Cuzzi G, Pinnetti C, Sampaolesi A, et al. Polyfunctional T-cells and effector memory phenotype are associated with active TB in HIV-infected patients. J Infect. 2014;69(6):533–45. https://doi.org/10.1016/j.jinf.2014.06.009.
45. Rozot V, Vigano S, Mazza-Stalder J, Idrizi E, Day CL, Perreau M, et al. Mycobacterium tuberculosis-specific CD8+ T cells are functionally and phenotypically different between latent infection and active disease. Eur J Immunol. 2013;43(6):1568–77. https://doi.org/10.1002/eji.201243262.
46. Nikolova M, Markova R, Drenska R, Muhtarova M, Todorova Y, Dimitrov V, et al. Antigen-specific CD4- and CD8-positive signatures in different phases of Mycobacterium tubercu-

losis infection. Diagn Microbiol Infect Dis. 2013;75(3):277–81. https://doi.org/10.1016/j.diagmicrobio.2012.11.023.
47. QuantiFERON-TB Gold Plus (QFT-Plus) ELISA Package Insert. Hilden: Qiagen; 2015.
48. Oxford Immunotec. T-SPOT.TB Package Insert US version 6. Available at https://www.T-SPOT.com/wp-content/uploads/2021/04/TB-PI-US-0001-V9.pdf. Accessed on 10/10/2018.
49. https://www.accessdata.fda.gov/cdrh_docs/pdf7/p070006b.pdf
50. Banach DB, Harris TG. Indeterminate QuantiFERON(R)-TB Gold results in a public health clinic setting. Int J Tuberc Lung Dis. 2011;15(12):1623–30. https://doi.org/10.5588/ijtld.11.0017.
51. Sharninghausen JC, Shapiro AE, Koelle DM, Kim HN. Risk factors for indeterminate outcome on interferon gamma release assay in non-US-born persons screened for latent tuberculosis infection. Open Forum Infect Dis. 2018;5(8):ofy184. https://doi.org/10.1093/ofid/ofy184.
52. Meier NR, Volken T, Geiger M, Heininger U, Tebruegge M, Ritz N. Risk factors for indeterminate interferon-gamma release assay for the diagnosis of tuberculosis in children—a systematic review and meta-analysis. Front Pediatr. 2019;7:208. https://doi.org/10.3389/fped.2019.00208.
53. Fabre V, Shoham S, Page KR, Shah M. High proportion of indeterminate QuantiFERON-TB gold in-tube results in an inpatient population is related to host factors and preanalytical steps. Open Forum Infect Dis. 2014;1(2):ofu088. https://doi.org/10.1093/ofid/ofu088.
54. Huang CT, Ruan SY, Tsai YJ, Kuo PH, Ku SC, Lee PL, et al. Effects of acute critical illnesses on the performance of interferon-gamma release assay. Sci Rep. 2016;6:19972. https://doi.org/10.1038/srep19972.
55. Mandalakas AM, Highsmith HY, Harris NM, Pawlicka A, Kirchner HL. T-SPOT.TB performance in routine pediatric practice in a low TB burden setting. Pediatr Infect Dis J. 2018;37(4):292–7. https://doi.org/10.1097/INF.0000000000001792.
56. Santin M, Munoz L, Rigau D. Interferon-gamma release assays for the diagnosis of tuberculosis and tuberculosis infection in HIV-infected adults: a systematic review and meta-analysis. PLoS One. 2012;7(3):e32482. https://doi.org/10.1371/journal.pone.0032482.
57. Banaei N, Gaur RL, Pai M. Interferon gamma release assays for latent tuberculosis: what are the sources of variability? J Clin Microbiol. 2016;54(4):845–50. https://doi.org/10.1128/JCM.02803-15.
58. Tagmouti S, Slater M, Benedetti A, Kik SV, Banaei N, Cattamanchi A, et al. Reproducibility of interferon gamma (IFN-gamma) release Assays. A systematic review. Ann Am Thorac Soc. 2014;11(8):1267–76. https://doi.org/10.1513/AnnalsATS.201405-188OC.
59. Metcalfe JZ, Cattamanchi A, McCulloch CE, Lew JD, Ha NP, Graviss EA. Test variability of the QuantiFERON-TB gold in-tube assay in clinical practice. Am J Respir Crit Care Med. 2013;187(2):206–11. https://doi.org/10.1164/rccm.201203-0430OC.
60. Yamasue M, Komiya K, Usagawa Y, Umeki K, Nureki SI, Ando M, et al. Factors associated with false negative interferon-gamma release assay results in patients with tuberculosis: a systematic review with meta-analysis. Sci Rep. 2020;10(1):1607. https://doi.org/10.1038/s41598-020-58459-9.
61. van Zyl-Smit RN, Zwerling A, Dheda K, Pai M. Within-subject variability of interferon-g assay results for tuberculosis and boosting effect of tuberculin skin testing: a systematic review. PLoS One. 2009;4(12):e8517. https://doi.org/10.1371/journal.pone.0008517.
62. Slater ML, Welland G, Pai M, Parsonnet J, Banaei N. Challenges with QuantiFERON-TB Gold assay for large-scale, routine screening of U.S. healthcare workers. Am J Respir Crit Care Med. 2013;188(8):1005–10. https://doi.org/10.1164/rccm.201305-0831OC.
63. Gao L, Lu W, Bai L, Wang X, Xu J, Catanzaro A, et al. Latent tuberculosis infection in rural China: baseline results of a population-based, multicentre, prospective cohort study. Lancet Infect Dis. 2015;15(3):310–9. https://doi.org/10.1016/S1473-3099(14)71085-0.
64. Gao L, Li X, Liu J, Wang X, Lu W, Bai L, et al. Incidence of active tuberculosis in individuals with latent tuberculosis infection in rural China: follow-up results of a population-based,

multicentre, prospective cohort study. Lancet Infect Dis. 2017;17(10):1053–61. https://doi.org/10.1016/S1473-3099(17)30402-4.
65. Abubakar I, Drobniewski F, Southern J, Sitch AJ, Jackson C, Lipman M, et al. Prognostic value of interferon-gamma release assays and tuberculin skin test in predicting the development of active tuberculosis (UK PREDICT TB): a prospective cohort study. Lancet Infect Dis. 2018;18(10):1077–87. https://doi.org/10.1016/S1473-3099(18)30355-4.
66. Andrews JR, Nemes E, Tameris M, Landry BS, Mahomed H, McClain JB, et al. Serial QuantiFERON testing and tuberculosis disease risk among young children: an observational cohort study. Lancet Respir Med. 2017;5(4):282–90. https://doi.org/10.1016/S2213-2600(17)30060-7.
67. Gupta RK, Lipman M, Jackson C, Sitch AJ, Southern J, Drobniewski F, et al. Quantitative IFN-gamma release assay and tuberculin skin test results to predict incident tuberculosis. A Prospective Cohort Study. Am J Respir Crit Care Med. 2020;201(8):984–91. https://doi.org/10.1164/rccm.201905-0969OC.
68. Winje BA, White R, Syre H, Skutlaberg DH, Oftung F, Mengshoel AT, et al. Stratification by interferon-gamma release assay level predicts risk of incident TB. Thorax. 2018; https://doi.org/10.1136/thoraxjnl-2017-211147.
69. Zellweger JP, Sotgiu G, Block M, Dore S, Altet N, Blunschi R, et al. Risk assessment of tuberculosis in contacts by IFN-gamma release assays. A Tuberculosis Network European Trials Group Study. Am J Respir Crit Care Med. 2015;191(10):1176–84. https://doi.org/10.1164/rccm.201502-0232OC.
70. Ledesma JR, Ma J, Zheng P, Ross JM, Vos T, Kyu HH. Interferon-gamma release assay levels and risk of progression to active tuberculosis: a systematic review and dose-response meta-regression analysis. BMC Infect Dis. 2021;21(1):467. https://doi.org/10.1186/s12879-021-06141-4.
71. Nemes E, Rozot V, Geldenhuys H, Bilek N, Mabwe S, Abrahams D, et al. Optimization and interpretation of serial QuantiFERON testing to measure acquisition of *Mycobacterium tuberculosis* infection. Am J Respir Crit Care Med. 2017;196(5):638–48. https://doi.org/10.1164/rccm.201704-0817OC.
72. Jonsson J, Westman A, Bruchfeld J, Sturegard E, Gaines H, Schon T. A borderline range for Quantiferon Gold In-Tube results. PLoS One. 2017;12(11):e0187313. https://doi.org/10.1371/journal.pone.0187313.
73. Lee MY, Kang SY, Lee WI, Kim MH. Introduction of a borderline range for the interpretation of QuantiFERON-TB gold In-tube results in Korean healthcare workers. Clin Lab. 2020;66(10) https://doi.org/10.7754/Clin.Lab.2020.200255.
74. Nienhaus A, Costa JT. Screening for tuberculosis and the use of a borderline zone for the interpretation of the interferon-gamma release assay (IGRA) in Portuguese healthcare workers. J Occup Med Toxicol. 2013;8(1):1. https://doi.org/10.1186/1745-6673-8-1.
75. Pollock NR, Kashino SS, Napolitano DR, Sloutsky A, Joshi S, Guillet J, et al. Evaluation of the effect of treatment of latent tuberculosis infection on QuantiFERON-TB gold assay results. Infect Control Hosp Epidemiol. 2009;30(4):392–5. https://doi.org/10.1086/596606.
76. Johnson JL, Geldenhuys H, Thiel BA, Toefy A, Suliman S, Pienaar B, et al. Effect of isoniazid therapy for latent TB infection on QuantiFERON-TB gold in-tube responses in adults with positive tuberculin skin test results in a high TB incidence area: a controlled study. Chest. 2014;145(3):612–7. https://doi.org/10.1378/chest.13-1232.
77. Adetifa IM, Ota MO, Jeffries DJ, Lugos MD, Hammond AS, Battersby NJ, et al. Interferon-gamma ELISPOT as a biomarker of treatment efficacy in latent tuberculosis infection: a clinical trial. Am J Respir Crit Care Med. 2013;187(4):439–45. https://doi.org/10.1164/rccm.201208-1352OC.
78. Zhang H, Xin H, Li X, Li H, Li M, Feng B, et al. Reversion of QuantiFERON-TB Gold In-Tube test in individuals with and without prophylactic treatment for latent tuberculosis infection: a systematic review and meta-analysis. J Infect. 2018;77(4):276–82. https://doi.org/10.1016/j.jinf.2018.04.009.

79. Clifford V, He Y, Zufferey C, Connell T, Curtis N. Interferon gamma release assays for monitoring the response to treatment for tuberculosis: a systematic review. Tuberculosis (Edinb). 2015;95(6):639–50. https://doi.org/10.1016/j.tube.2015.07.002.
80. American Academy of Pediatrics. Committee on Infectious Diseases. Red book: 2021 report of the Committee on Infectious Diseases. 32nd ed. Elk Grove Village, IL: American Academy of Pediatrics; 2021.
81. Ahmed A, Feng PI, Gaensbauer JT, Reves RR, Khurana R, Salcedo K, et al. Interferon-gamma release assays in children <15 years of age. Pediatrics. 2020;145(1):e20191930. https://doi.org/10.1542/peds.2019-1930.
82. Malhame I, Cormier M, Sugarman J, Schwartzman K. Latent tuberculosis in pregnancy: a systematic review. PLoS One. 2016;11(5):e0154825. https://doi.org/10.1371/journal.pone.0154825.
83. Mathad JS, Bhosale R, Sangar V, Mave V, Gupte N, Kanade S, et al. Pregnancy differentially impacts performance of latent tuberculosis diagnostics in a high-burden setting. PLoS One. 2014;9(3):e92308. https://doi.org/10.1371/journal.pone.0092308.
84. Mathad JS, Bhosale R, Balasubramanian U, Kanade S, Mave V, Suryavanshi N, et al. Quantitative IFN-gamma and IL-2 response associated with latent tuberculosis test discordance in HIV-infected pregnant women. Am J Respir Crit Care Med. 2016;193(12):1421–8. https://doi.org/10.1164/rccm.201508-1595OC.
85. LaCourse SM, Cranmer LM, Matemo D, Kinuthia J, Richardson BA, Horne DJ, et al. Effect of pregnancy on interferon gamma release assay and tuberculin skin test detection of latent TB infection among HIV-infected women in a high burden setting. J Acquir Immune Defic Syndr. 2017;75(1):128–36. https://doi.org/10.1097/QAI.0000000000001298.
86. Sosa LE, Njie GJ, Lobato MN, Bamrah Morris S, Buchta W, Casey ML, et al. Tuberculosis screening, testing, and treatment of U.S. health care personnel: recommendations from the National Tuberculosis Controllers Association and CDC, 2019. MMWR Morb Mortal Wkly Rep. 2019;68(19):439–43. https://doi.org/10.15585/mmwr.mm6819a3.
87. Pettit AC, Stout JE, Belknap R, Benson CA, Seraphin MN, Lauzardo M, et al. Optimal testing choice and diagnostic strategies for latent tuberculosis infection among U.S.-born people living with HIV. Clin Infect Dis. 2020; https://doi.org/10.1093/cid/ciaa1135.
88. Campbell JR, Winters N, Menzies D. Absolute risk of tuberculosis among untreated populations with a positive tuberculin skin test or interferon-gamma release assay result: systematic review and meta-analysis. BMJ. 2020;368:m549. https://doi.org/10.1136/bmj.m549.
89. Theodoropoulos N, Lanternier F, Rassiwala J, McNatt G, Preczewski L, DeMayo E, et al. Use of the QuantiFERON-TB Gold interferon-gamma release assay for screening transplant candidates: a single-center retrospective study. Transpl Infect Dis. 2012;14(1):1–8. https://doi.org/10.1111/j.1399-3062.2011.00666.x.
90. Nasiri MJ, Pormohammad A, Goudarzi H, Mardani M, Zamani S, Migliori GB, et al. Latent tuberculosis infection in transplant candidates: a systematic review and meta-analysis on TST and IGRA. Infection. 2019;47(3):353–61. https://doi.org/10.1007/s15010-019-01285-7.
91. Ferguson TW, Tangri N, Macdonald K, Hiebert B, Rigatto C, Sood MM, et al. The diagnostic accuracy of tests for latent tuberculosis infection in hemodialysis patients: a systematic review and meta-analysis. Transplantation. 2015;99(5):1084–91. https://doi.org/10.1097/TP.0000000000000451.
92. Horne DJ, Narita M, Spitters CL, Parimi S, Dodson S, Limaye AP. Challenging issues in tuberculosis in solid organ transplantation. Clin Infect Dis. 2013;57(10):1473–82. https://doi.org/10.1093/cid/cit488.
93. Subramanian A, Dorman S, Practice ASTIDCo. Mycobacterium tuberculosis in solid organ transplant recipients. Am J Transplant. 2009;9(Suppl 4):S57–62. https://doi.org/10.1111/j.1600-6143.2009.02894.x.
94. https://www.ecdc.europa.eu/en/publications-data/programmatic-management-latent-tuberculosis-infection-european-union

95. Ruhwald M, Aggerbeck H, Gallardo RV, Hoff ST, Villate JI, Borregaard B, et al. Safety and efficacy of the C-Tb skin test to diagnose Mycobacterium tuberculosis infection, compared with an interferon γ release assay and the tuberculin skin test: a phase 3, double-blind, randomised, controlled trial. Lancet Respir Med. 2017;5(4):259–68. https://doi.org/10.1016/s2213-2600(16)30436-2.
96. Slogotskaya L, Bogorodskaya E, Ivanova D, Sevostyanova T. Comparative sensitivity of the test with tuberculosis recombinant allergen, containing ESAT6-CFP10 protein, and Mantoux test with 2 TU PPD-L in newly diagnosed tuberculosis children and adolescents in Moscow. PLoS One. 2018;13(12):e0208705. https://doi.org/10.1371/journal.pone.0208705.
97. Li F, Xu M, Qin C, Xia L, Xiong Y, Xi X, et al. Recombinant fusion ESAT6-CFP10 immunogen as a skin test reagent for tuberculosis diagnosis: an open-label, randomized, two-centre phase 2a clinical trial. Clin Microbiol Infect. 2016;22(10):889.e9–889.e16. https://doi.org/10.1016/j.cmi.2016.07.015.
98. Badaro R, Machado BAS, Duthie MS, Araujo-Neto CA, Pedral-Sampaio D, Nakatani M, et al. The single recombinant M. tuberculosis protein DPPD provides enhanced performance of skin testing among HIV-infected tuberculosis patients. Amb Express. 2020;10(1):133. https://doi.org/10.1186/s13568-020-01068-6.
99. Krutikov M, Faust L, Nikolayevskyy V, Hamada Y, Gupta RK, Cirillo D, et al. The diagnostic performance of novel skin-based in-vivo tests for tuberculosis infection compared with purified protein derivative tuberculin skin tests and blood-based in vitro interferon-γ release assays: a systematic review and meta-analysis. Lancet Infect Dis. 2021;22:250–64. https://doi.org/10.1016/s1473-3099(21)00261-9. (Eur Respir J 37 2011)
100. Aggerbeck H, Giemza R, Joshi P, Tingskov PN, Hoff ST, Boyle J, et al. Randomised clinical trial investigating the specificity of a novel skin test (C-Tb) for diagnosis of *M. tuberculosis* Infection. PLoS One. 2013;8(5):e64215. https://doi.org/10.1371/journal.pone.0064215.
101. Bella CD, Spinicci M, Alnwaisri HFM, Bartalesi F, Tapinassi S, Mencarini J, et al. LIOFeron®TB/LTBI: a novel and reliable test for LTBI and tuberculosis. Int J Infect Dis. 2020;91:177–81. https://doi.org/10.1016/j.ijid.2019.12.012.
102. Dong Y, Demaria S, Sun X, Santori FR, Jesdale BM, Groot ASD, et al. HLA-A2-restricted CD8 + -cytotoxic-T-cell responses to novel epitopes in *Mycobacterium tuberculosis* superoxide dismutase, alanine dehydrogenase, and glutamine synthetase. Infect Immun. 2004;72(4):2412–5. https://doi.org/10.1128/iai.72.4.2412-2415.2004.
103. Serra-Vidal MM, Latorre I, Franken KLCM, Díaz J, Souza-Galvão MLD, Casas I, et al. Immunogenicity of 60 novel latency-related antigens of *Mycobacterium tuberculosis*. Front Microbiol. 2014;5:517. https://doi.org/10.3389/fmicb.2014.00517.
104. Coppola M, Meijgaarden KEV, Franken KLMC, Commandeur S, Dolganov G, Kramnik I, et al. New genome-wide algorithm identifies novel in-vivo expressed *Mycobacterium tuberculosis* antigens inducing human T-cell responses with classical and unconventional cytokine profiles. Sci Rep. 2016;6(1):37793. https://doi.org/10.1038/srep37793.
105. Meier NR, Jacobsen M, Ottenhoff THM, Ritz N. A systematic review on novel *Mycobacterium tuberculosis* antigens and their discriminatory potential for the diagnosis of latent and active tuberculosis. Front Immunol. 2018;9:2476. https://doi.org/10.3389/fimmu.2018.02476.
106. Latorre I, Domínguez J. Dormancy antigens as biomarkers of latent tuberculosis infection. Ebiomedicine. 2015;2(8):790–1. https://doi.org/10.1016/j.ebiom.2015.06.017.
107. Ruhwald M, Thurah LD, Kuchaka D, Zaher MR, Salman AM, Abdel-Ghaffar A-R, et al. Introducing the ESAT-6 free IGRA, a companion diagnostic for TB vaccines based on ESAT-6. Sci Rep. 2017;7(1):45969. https://doi.org/10.1038/srep45969.
108. Villar-Hernandez R, Blauenfeldt T, Garcia-Garcia E, Muriel-Moreno B, De Souza-Galvao ML, Millet JP, et al. Diagnostic benefits of adding EspC, EspF and Rv2348-B to the QuantiFERON Gold In-tube antigen combination. Sci Rep. 2020;10(1):13234. https://doi.org/10.1038/s41598-020-70204-w.
109. Sudbury EL, Clifford V, Messina NL, Song R, Curtis N. Mycobacterium tuberculosis-specific cytokine biomarkers to differentiate active TB and LTBI: a systematic review. J Infect. 2020;81(6):873–81. https://doi.org/10.1016/j.jinf.2020.09.032.

110. Ruhwald M, Bjerregaard-Andersen M, Rabna P, Eugen-Olsen J, Ravn P. IP-10, MCP-1, MCP-2, MCP-3, and IL-1RA hold promise as biomarkers for infection with *M. tuberculosis* in a whole blood based T-cell assay. BMC Res Notes. 2009;2(1):19. https://doi.org/10.1186/1756-0500-2-19.
111. Ruhwald M, Aabye MG, Ravn P. IP-10 release assays in the diagnosis of tuberculosis infection: current status and future directions. Expert Rev Mol Diagn. 2014;12(2):175–87. https://doi.org/10.1586/erm.11.97.
112. Aabye MG, Ruhwald M, PrayGod G, Jeremiah K, Faurholt-Jepsen M, Faurholt-Jepsen D, et al. Potential of interferon—inducible protein 10 in improving tuberculosis diagnosis in HIV-infected patients. Eur Respir J. 2010;36(6):1488–90. https://doi.org/10.1183/09031936.00039010.
113. Aabye MG, Latorre I, Diaz J, Maldonado J, Mialdea I, Eugen-Olsen J, et al. Dried plasma spots in the diagnosis of tuberculosis: IP-10 release assay on filter paper. Eur Respir J. 2013;42(2):495–503. https://doi.org/10.1183/09031936.00129412.
114. Villar-Hernandez R, Latorre I, De Souza-Galvao ML, Jimenez MA, Ruiz-Manzano J, Pilarte J, et al. Use of IP-10 detection in dried plasma spots for latent tuberculosis infection diagnosis in contacts via mail. Sci Rep. 2019;9(1):3943. https://doi.org/10.1038/s41598-019-40778-1.
115. Robison HM, Escalante P, Valera E, Erskine CL, Auvil L, Sasieta HC, et al. Precision immunoprofiling to reveal diagnostic signatures for latent tuberculosis infection and reactivation risk stratification. Integr Biol. 2019;11(1):zyz001. https://doi.org/10.1093/intbio/zyz001.
116. Wu J, Wang S, Lu C, Shao L, Gao Y, Zhou Z, et al. Multiple cytokine responses in discriminating between active tuberculosis and latent tuberculosis infection. Tuberculosis (Edinb). 2017;102:68–75. https://doi.org/10.1016/j.tube.2016.06.001.
117. Robison HM, Chapman CA, Zhou H, Erskine CL, Theel E, Peikert T, et al. Risk assessment of latent tuberculosis infection through a multiplexed cytokine biosensor assay and machine learning feature selection. Sci Rep. 2021;11(1):20544. https://doi.org/10.1038/s41598-021-99754-3.
118. Steingart KR, Henry M, Laal S, Hopewell PC, Ramsay A, Menzies D, et al. A systematic review of commercial serological antibody detection tests for the diagnosis of extrapulmonary tuberculosis. Thorax. 2007;62(10):911. https://doi.org/10.1136/thx.2006.075754.
119. Kunnath-Velayudhan S, Salamon H, Wang H-Y, Davidow AL, Molina DM, Huynh VT, et al. Dynamic antibody responses to the Mycobacterium tuberculosis proteome. Proc Natl Acad Sci. 2010;107(33):14703–8. https://doi.org/10.1073/pnas.1009080107.
120. Maekura R, Kitada S, Osada-Oka M, Tateishi Y, Ozeki Y, Fujicawa T, et al. Serum antibody profiles in individuals with latent *Mycobacterium tuberculosis* infection. Microbiol Immunol. 2019;63(3-4):130–8. https://doi.org/10.1111/1348-0421.12674.
121. Pathakumari B, Anbarasu D, Parthasarathy RT, Raja A. PpiA antigen specific immune response is a potential biomarker for latent tuberculosis infection. Tuberculosis. 2015;95(6):736–43. https://doi.org/10.1016/j.tube.2015.07.006.
122. Wang S, Wu J, Chen J, Gao Y, Zhang S, Zhou Z, et al. Evaluation of Mycobacterium tuberculosis-specific antibody responses for the discrimination of active and latent tuberculosis infection. Int J Infect Dis. 2018;70:1–9. https://doi.org/10.1016/j.ijid.2018.01.007.
123. Walzl G, Ronacher K, Hanekom W, Scriba TJ, Zumla A. Immunological biomarkers of tuberculosis. Nat Rev Immunol. 2011;11(5):343–54. https://doi.org/10.1038/nri2960.
124. Maertzdorf J, Repsilber D, Parida SK, Stanley K, Roberts T, Black G, et al. Human gene expression profiles of susceptibility and resistance in tuberculosis. Genes Immun. 2011;12(1):15–22. https://doi.org/10.1038/gene.2010.51.
125. Mistry R, Cliff JM, Clayton CL, Beyers N, Mohamed YS, Wilson PA, et al. Gene-expression patterns in whole blood identify subjects at risk for recurrent tuberculosis. J Infect Dis. 2007;195(3):357–65. https://doi.org/10.1086/510397.
126. Gupta RK, Turner CT, Venturini C, Esmail H, Rangaka MX, Copas A, et al. Concise whole blood transcriptional signatures for incipient tuberculosis: a systematic review and patient-level pooled meta-analysis. Lancet Respir Med. 2020;8(4):395–406. https://doi.org/10.1016/s2213-2600(19)30282-6.

127. Suliman S, Thompson EG, Sutherland J, Weiner J, Ota MOC, Shankar S, et al. Four-gene pan-African blood signature predicts progression to tuberculosis. Am J Resp Crit Care. 2018;197(9):1198–208. https://doi.org/10.1164/rccm.201711-2340oc.
128. Roe J, Venturini C, Gupta RK, Gurry C, Chain BM, Sun Y, et al. Blood transcriptomic stratification of short-term risk in contacts of tuberculosis. Clin Infect Dis. 2019;70(5):ciz252. https://doi.org/10.1093/cid/ciz252.
129. Scriba TJ, Fiore-Gartland A, Penn-Nicholson A, Mulenga H, Mbandi SK, Borate B, et al. Biomarker-guided tuberculosis preventive therapy (CORTIS): a randomised controlled trial. Lancet Infect Dis. 2021;21(3):354–65. https://doi.org/10.1016/s1473-3099(20)30914-2.
130. Wejse C. Transcriptomic signatures have a place in short-term prediction of incident tuberculosis. Lancet Infect Dis. 2021;21(3):299–300. https://doi.org/10.1016/S1473-3099(20)30980-4.
131. Gupta RK, Noursadeghi M. Blood transcriptomic biomarkers for tuberculosis screening: time to redefine our target populations? Lancet Global Heal. 2021;9(6):e736–e7. https://doi.org/10.1016/s2214-109x(21)00088-7.

Chapter 10
Advances in Treatment of Latent TB Infection: What Is the Latest Approach to Treat Latent TB Infection to Prevent Pulmonary TB?

Bijan Ghassemieh and Masahiro Narita

Abstract An estimated one-quarter of the world population has latent TB infection (LTBI) and 5–10% of those will develop TB disease during their lifetime. Treatment of LTBI can decrease the risk of developing TB disease by 60–90%. Historically, daily isoniazid (INH) for 6–9 months was commonly used to treat LTBI, but this regimen has a relatively high risk of drug-induced liver injury and a low rate of treatment completion. Rifamycin-based regimens (3 months of once weekly INH and rifapentine, 4 months of daily rifampin, and 3 months of daily INH and rifampin) are now preferred because of their effectiveness, safety, and high treatment completion rates. Pretreatment evaluation includes ruling out TB disease as well as assessing the risk for adverse effects and drug–drug interactions. A regimen of 4 months of daily rifampin (4R) is a preferred treatment because of a lower rate of treatment discontinuation, a lower rate of hepatotoxicity and a higher rate of treatment completion, but no evidence is available for its effectiveness in HIV-positive persons. A regimen of 3 months of once weekly INH plus rifapentine (3HP) is also a preferred treatment for adults and children aged >2 years, including HIV-positive persons. However, 3HP is associated with a systemic drug reaction including syncope and hypotension and more discontinuation because of adverse effects.

Keywords Latent TB infection · Rifampin · Rifapentine

B. Ghassemieh (✉) · M. Narita
Division of Pulmonary, Critical Care and Sleep Medicine, University of Washington, Seattle, WA, USA

TB Control Program, Public Health – Seattle and King County, Seattle, WA, USA
e-mail: bijang@uw.edu

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1 Introduction

1.1 The Spectrum of TB Manifestations in Humans

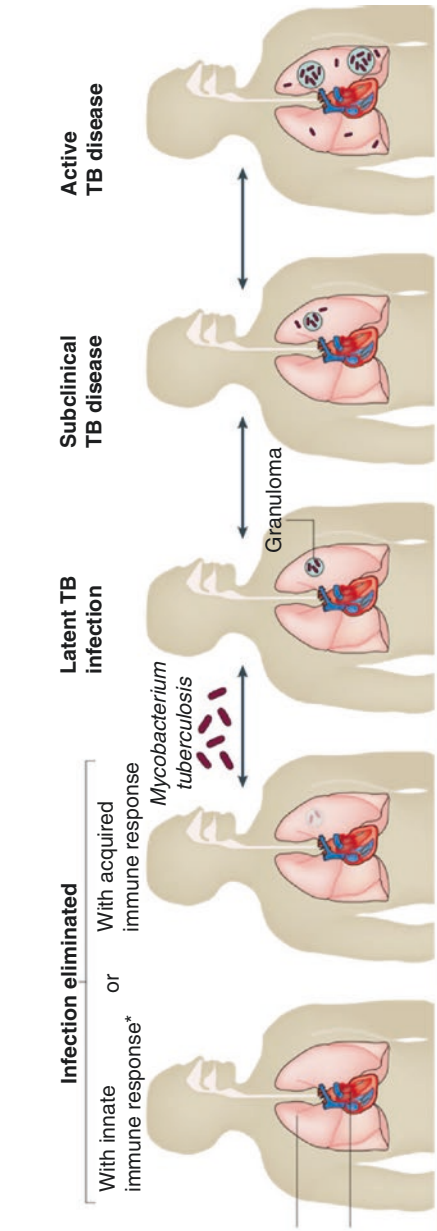
Mycobacterium tuberculosis infection in humans has historically been thought to have two distinct forms, with TB disease-causing symptoms, radiographic abnormalities, and positive cultures from clinical specimens, and latent TB infection (LTBI) representing an asymptomatic and culture-negative state of equilibrium between semidormant TB bacilli and the human immune response. While this distinction is helpful clinically, it is now thought that *M. tuberculosis* infection has multiple stages in humans across a clinical spectrum, ranging from clearance of infection by the innate or acquired immune system, latent infection, subclinical TB disease, and overt active TB disease (see Fig. 10.1) [1].

With currently available tools, it is difficult to differentiate between LTBI and subclinical disease, and impossible to differentiate between infection cleared by the acquired immune response and latent TB infection. Nevertheless, clinical care and public health practice necessitate practical definitions for LTBI. For the purposes of clinical and programmatic management, LTBI is defined as:

- Evidence of cell-mediated immunity to TB antigens (either a positive tuberculin skin test or interferon-gamma release assay).
- No clinical signs or symptoms of TB disease.
- No radiographic signs of TB disease.

1.2 Rationale For LTBI Treatment

An estimated one-quarter of the world population has LTBI [2]. This represents a vast reservoir of potential future disease activation and transmission. On an individual level, treatment of LTBI can decrease the risk of TB disease by 60–90% [3]. On a population level, since human hosts make up the vast majority of the world's reservoir of organisms that cause TB disease [4, 5], LTBI treatment offers an opportunity to move toward TB elimination by decreasing that reservoir. In fact, after isolation and treatment of infectious TB cases, the World Health Organization identifies LTBI treatment in those with a high risk of progression to TB disease ("TB reactivation") as the next most important intervention to decrease TB incidence [6]. This is especially true in low-burden TB settings, where reactivation of LTBI accounts for the majority of TB disease [7]. Conversely, in settings where the prevalence of TB disease is very high with high transmission rates, LTBI therapy may not offer long-lasting benefits because of repeated TB exposure and reinfection [8].



	Infection eliminated		Latent TB infection	Subclinical TB disease	Active TB disease
TST	Negative	Positive	Positive	Positive	Usually positive
IGRA	Negative	Positive	Positive	Positive	Usually positive
Culture	Negative	Negative	Negative	Intermittently positive	Positive
Sputum smear	Negative	Negative	Negative	Usually negative	Positive or negative
Infectious	No	No	No	Sporadically	Yes
Symptoms	None	None	None	Mild or none	Mild to severe
Preferred treatment	None	None	Preventive therapy	Multidrug therapy	Multidrug therapy

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Fig. 10.1 The spectrum of TB—from *Mycobacterium tuberculosis* infection to active (pulmonary) TB disease (used with permission from [1])

1.3 Content and Focus of This Chapter

This chapter will discuss who to target for LTBI treatment, how to evaluate a patient prior to initiating LTBI treatment, the various recommended LTBI treatment regimens, and LTBI treatment in select special situations. It should be noted that multiple different bodies have published guidelines for the treatment of LTBI, including the WHO [6], the United States Centers for Disease Control and Prevention (CDC), and the National Tuberculosis Controllers Association (NTCA) [3], and multiple other public health organizations [9]. These guidelines are all in agreement that certain high-risk groups should be targeted for LTBI treatment, but the specific groups recommended for targeting and the specific treatment regimens recommended differ based on local TB epidemiology, local epidemiology of specific risk factors for progression to TB disease (for example, local HIV prevalence), and local healthcare infrastructure and resources. This chapter will focus primarily on current practice in the United States, as this is the authors' area of expertise. The reader is encouraged to review the WHO guidelines and their local jurisdiction guidelines for more information.

2 Targeting LTBI Testing and Therapy

Most people with LTBI will never develop TB disease. In fact, only 5–10% of those with LTBI will develop TB disease in their lifetime [6, 10, 11]. For the other 90–95%, LTBI therapy would incur potential toxicities and cost with no benefit. Unfortunately, there is no currently available biomarker that can accurately predict which patients with LTBI will develop TB disease, although this is an area of active research [12]. Therefore, clinicians and public health programs must rely on established epidemiologic and clinical risk factors to identify patients at increased risk for TB disease that would benefit from LTBI treatment. These risk factors can be broadly divided into those that increase risk for TB infection, and those that increase risk for progression to active TB disease if infected.

Tables 10.1 and 10.2, adapted from reference [13], provide estimates for some common specific risk factors for TB disease in the United States. Notably, recent contact with an infectious TB case is not only a risk factor for TB infection, but also is a significant risk factor for progression to TB disease in the 2–3 years after acquisition of TB infection. While different organizations recommend prioritization of different groups for LTBI interventions based on differences in local TB epidemiology and resources, there is a wide consensus that the following groups should be prioritized: people living with HIV (PLHIV); close contacts to infectious TB cases; patients initiating anti-TNF-alpha therapy, chemotherapy, or planning organ transplantation; patients with end-stage renal disease on hemodialysis; patients with silicosis; and patients with recent conversion on an LTBI test (indicating recent infection) [6, 9, 14, 15]. In addition, most but not all organizations recommend

Table 10.1 Estimated prevalence of latent TB infection, United States

Group	Expected prevalence[a], % (95% confidence interval)
Born outside the United States	18.7 (13.5–25.2)
Close contacts of persons with infectious TB	37.1 (35.7–38.5)
Persons experiencing homelessness	12.8 (12.2–13.5) 32.4 (30.5–34.4)
Injection-drug users	16.1 (12.5–22.4) 27.7 (19.3–37.5) 22.4 (17.7–28.5) 14.0 (11.4–17.1)
Prisoners	17.0 (16.8–17.1)
US-born, No other risk factors	1.8 (1.4–2.1)

Adapted from reference [13]
[a]Note that some groups have more than one prevalence estimate, each taken from a different study

Table 10.2 Risk factors for progression from latent TB infection to TB disease

Risk factor	Relative risk[a] (95% confidence interval)
Advanced, untreated HIV	9.9 (8.7–11) 9.5 (3.6–25)
Close contact with an infectious TB case[b]	6.1 (5.5–6.8)
Old, healed TB on chest imaging (not previously treated)	5.2 (3.4–8.0)
Treatment with >= 15 mg Prednisone per day for 2 weeks or more	2.8 (1.7–4.6)
Chronic renal failure	2.4 (2.1–2.8)
Treatment with TNF-alpha inhibitor	2.0 (1.1–3.5)
Poorly controlled diabetes	1.7 (1.5–2.2)

Adapted from reference [13]
Relative risk was calculated compared to an individual with no risk factors
[a]Note that some risk factors have more than one relative risk estimate, each taken from a different study
[b]Relative risk was calculated for the first 3 years after exposure

prioritizing patients with fibronodular changes on chest imaging consistent with "old healed TB" if they have not been previously treated for TB disease. For other risk factors, a useful online tool published by McGill University can estimate an individual's risk of TB disease up to age 80 years based on certain clinical characteristics [16]. Note that for societies with large proportions of individuals living beyond 80 years old, this tool may underestimate lifetime risk.

Finally, in some instances, the decision regarding initiation of LTBI treatment in a particular individual also needs to take into account the risk of disease transmission to others should that individual develop infectious TB. For example, if the individual lives in a congregate setting with potential for secondary TB cases (i.e., a homeless shelter), a clinician or public health program might consider treatment if

there is otherwise clinical equipoise. Similarly, if the person has high-risk household members (i.e., a young child or immunosuppressed individual), treatment should be more strongly considered.

3 Pretreatment Evaluation

3.1 Ruling Out TB Disease

It is imperative that TB disease is ruled out before LTBI therapy is started, as the 1–2 drug regimens used for LTBI may lead to drug resistance if used for TB disease. Operationally, ruling out TB disease is done with a standard symptom screen, physical examination, and chest X-ray. If results are abnormal, sputum or an appropriate specimen depending on the location of the abnormalities is collected and sent for smear, culture, and PCR testing for the *M. tuberculosis* (MTB) complex. If the suspicion for TB disease is moderate or high, the clinician should consider empiric multidrug therapy for TB disease while awaiting test results. Ruling out TB disease in immunosuppressed or pediatric patients can be particularly challenging, as extrapulmonary TB disease, subclinical pulmonary disease, and atypical TB manifestations on chest X-rays are all common [17–19].

3.2 Assessing Risk for Adverse Effects

Hepatoxicity is the most important and potentially severe adverse effect to monitor for with LTBI treatment. The main risk factors for hepatoxicity include age, preexisting liver disease, other concurrent hepatoxic medications, alcohol abuse, pregnancy or the post-partum 3-month period, and HIV [20]. As described below, regimens that include isoniazid (INH) are more hepatotoxic [3].

Peripheral neuropathy from INH is more common with advanced age, malnutrition, diabetes mellitus, renal failure, alcoholism, pregnancy, breastfeeding, and HIV [21]. For those at risk of INH-associated peripheral neuropathy, pyridoxine can be effective in preventing this.

3.3 Evaluating for Potential Drug Interactions

Rifamycins are strong inducers of the hepatic cytochrome P450 system, which can lead to significant decreases in the blood concentration and clinical effectiveness of many other drugs. Commonly encountered rifamycin drug interactions include interactions with some oral contraceptives, anticoagulants, antihypertensives,

anticonvulsants, antiretroviral medications, opiates (including methadone), and opiate analogs [22]. The reader is encouraged to consult an online drug interaction tool when prescribing rifamycins.

3.4 Other Important Pretreatment Considerations

For every patient starting LTBI therapy, it is important to assess barriers to adherence, such as poor health literacy, drug or alcohol abuse, or unstable housing. Potential interventions to address barriers to adherence include assigning a case manager, providing incentives for treatment completion, or administering directly observed LTBI treatment.

For contacts of infectious TB cases, the source case's drug susceptibility profile should be reviewed and medication adjustments made accordingly.

4 Drug Regimens to Treat LTBI

Recommended regimens to treat LTBI include isoniazid and rifapentine given once weekly for 12 weeks (3HP), 3–4 months of rifampin given daily (3R or 4R), or 3 months of INH and rifampin given daily (3HR). Six months of INH given daily (6H) or 9 months of INH given daily (9H) are considered acceptable but not preferred regimens. The reader is referred to reference [3] for specific dosing recommendations for each regimen.

These regimens can be used in any patient with LTBI with a few exceptions. There is limited trial data evaluating 3R or 4R in PLHIV [3], although based on programmatic experience this is still an option if other regimens are not feasible or tolerated. In addition, 3HP is not recommended for children aged <2 years or pregnant women due to limited data regarding efficacy and safety in these groups [3].

4.1 LTBI Treatment History

In the 1950s and 1960s, multiple randomized controlled trials (RCTs) conducted involving over 100,000 patients showed that on average INH had a 60% effectiveness in preventing TB disease, and a 90% effectiveness in those that actually completed treatment [21]. Most of these trials included a duration of INH of 6 or 12 months. After a reanalysis of data from multiple trials, it was determined that 9 months of INH was the optimal duration that best balanced TB disease prevention and medication toxicity [23]. Since then, INH of varying durations has been recommended as a first-line treatment for LTBI.

More recently, 3 months of Isoniazid and Rifampin (3HR) and 4 months of Rifampin (4R) have been recommended by some national and international LTBI treatment guidelines based on programmatic experience and small trials suggesting similar effectiveness and toxicity to INH [24–26].

In the last 10 years, two large RCTs evaluating rifamycin-based regimens have provided significant data to support the use of rifamycin-based regimens for LTBI treatment. The first, published in 2011, randomized 7731 subjects with LTBI at increased risk of progression to TB disease to 12 weekly doses of INH and Rifapentine (a rifamycin with increased in vitro activity against MTB and a longer half-life than Rifampin) vs. 9 months of INH daily. The regimen had similar effectiveness, better completion rates, less hepatotoxicity, and a slightly higher rate of discontinuation due to adverse effects compared to INH [27]. The slightly higher rate of discontinuation was due to a flu-like syndrome that is discussed further below. While 3HP was initially only recommended for directly observed therapy, based on a large trial that showed similar rates of treatment completion and side effects in patients aged >= 18 years in the United States [28], the US CDC now recommends that 3HP may be given self-administered [29].

The second large RCT, published in 2018, randomized 6859 subjects with LTBI at increased risk of progression to TB disease to 4 months of rifampin daily (4R) or 9 months of INH daily. 4R had similar effectiveness, better completion rates, and less toxicity [30].

Based on these studies and follow-up studies of rifamycin-based regimens in other populations, including pediatric populations [31, 32] and PLHIV [27, 33], rifamycin-based regimens are now recommended as the preferred regimens by the US CDC and NTCA based on similar efficacy, decreased rates of hepatotoxicity, and increased rates of treatment completion compared to INH [3].

4.2 Efficacy and Hepatotoxicity of Each LTBI Regimen

In 2018, the US CDC and NTCA conducted a network meta-analysis of 63 randomized controlled trials to determine the effectiveness and hepatotoxicity of LTBI regimens [3]. The findings of this meta-analysis are summarized in Table 10.3. As is shown in the table, rifamycin-based regimens have similar or improved efficacy and less hepatotoxicity compared to INH.

4.3 Adverse Effects

As discussed above, hepatotoxicity can be seen with all LTBI regimens, but is more common with INH-based regimens. It can be challenging to differentiate drug-induced liver injury from what is termed “hepatic adaptation” to INH. In hepatic

Table 10.3 Efficacy and hepatoxicity For LTBI regimens

Regimen	Abbreviation	Odds ratio (95% credible interval) for TB disease[a]	Odds ratio (95% credible interval) for hepatotoxicity[a]
3 months of weekly INH and Rifapentine	3HP	0.36 (0.18–0.72)	0.53 (0.13–2.13)
3–4 months of Rifampin daily	3R, 4R	0.25 (0.12–0.50)	0.13 (<0.02–0.72)
3 months of INH and Rifampin daily	3HR	0.33 (0.20–0.53)	0.73 (0.22–2.38)
6 months of INH daily	6INH	0.40 (0.26–0.59)	1.11 (0.41–3.15)
9 months of INH daily	9INH	0.47 (0.24–0.90)	1.77 (0.35–8.32)

Adapted from reference [3]
[a]Compared to no treatment

adaptation, there is an asymptomatic, transient elevations in ALT that represent mild hepatocellular injury accompanied by physiologic adaptive responses to drug exposure that are thought to protect against further hepatocellular injury. Guidelines recommend discontinuing or switching LTBI therapy if hepatic aminotransferases are greater than 3 times the upper limit of normal in a patient with symptoms of liver injury and 5 times the upper limit of normal in an asymptomatic patient [20].

INH can cause peripheral neuropathy as discussed previously. Gastrointestinal upset and rash are relatively common adverse effects that can be caused by any of the TB regimens. Rifamycins cause orange discoloration of body fluids that is not dangerous and goes away once therapy is completed.

It is important to note that in the largest trial of 3HP vs. INH, while the rate of hepatotoxicity was less with 3HP, the overall rate of discontinuation due to side effects was higher [34, 35]. 3.5% of patients had a significant systemic drug reaction with 3HP, compared to just 0.4% with 9INH. The majority of the systemic drug reactions were a flu-like syndrome (with possible symptoms including lightheadedness, dizziness, headache, nausea/vomiting, dyspnea, angioedema, rash, and syncope), and 0.3% of subjects receiving 3HP had severe reactions (a composite end point that included hospitalization, hypotension, loss of consciousness, and/or grade 4 adverse event). This reaction is not well understood and is difficult to predict [35].

Some other very rare but severe adverse effects to be aware of include hypersensitivity reactions to INH (manifesting as systemic symptoms and urticaria), drug-induced lupus from INH, hypersensitivity reactions to rifampin (manifesting as rash, fever, and hepatosplenomegaly), renal failure from rifampin (through a variety of mechanisms), and rifampin related immune thrombocytopenia or anemia [36].

A full summary of all possible side effects is outside the scope of this chapter. The reader is referred to an excellent, free resource that provides medication fact sheets for all TB medications with lists of potential side effects [37].

5 LTBI Therapy in Special Situations

5.1 HIV and LTBI

Treatment of LTBI in PLHIV is an important, large, and complicated topic. A full discussion is outside the scope of this chapter and consultation with an expert in HIV is advised. However, there are a few key concepts that are worth emphasizing. First, as mentioned previously, severely immunosuppressed patients may have minimal or atypical TB disease symptoms, are at higher risk for extrapulmonary disease, and may have atypical or normal chest X-ray patterns despite having TB disease. The clinician needs to maintain a high index of suspicion for TB disease and rule this out prior to starting LTBI treatment. Second, immunosuppression can lead to false-negative TST or IGRA results. For this reason, empiric LTBI therapy should be considered for PLHIV with close contact to an infectious TB case regardless of LTBI testing results. Conversely, the benefits of LTBI treatment in HIV have only been associated with decreased TB incidence in those with a positive test for LTBI at baseline [38]. Third, rifamycins can interact with antiretroviral therapy, especially protease inhibitors. Finally, there is limited data to support the use of 4R in PLHIV [3]. 3HP may be a better rifamycin-based option, as this has been demonstrated to have similar efficacy and toxicity compared to 9INH [3, 27, 33].

5.2 Pediatric LTBI

Children under the age of 5 years with LTBI are at increased risk for progression to TB disease, including disseminated TB, central nervous system TB, and mortality. This risk is especially high in the first year of life [39]. As with HIV, young children with TB disease can have atypical clinical presentations and imaging because the proportion of those with extrapulmonary or disseminated TB is higher than adults, and care needs to be taken to rule out TB disease prior to starting LTBI treatment.

Thankfully, the risk of hepatitis with LTBI treatment is very low [21]. Children aged <2 years should not receive 3HP due to limited data regarding safety and efficacy, and the dosing for other regimens should be adjusted [3]. Otherwise, the treatment and management are similar to adults.

5.3 Window Prophylaxis

After exposure to an infectious TB case, it can take up to 8 weeks for a TST or IGRA to turn positive [40, 41]. As a result, for close contacts who are at high risk of severe and rapidly progressive TB disease (i.e., increased risk of central nervous system TB or death), the US CDC and NTCA recommend initiating “window

prophylaxis" once TB disease has been ruled out. In children <5 years old and immunocompromised close contacts, if the initial LTBI test is negative and TB disease is ruled out by history, physical exam, and chest X-ray, empiric LTBI treatment is initiated. The contact is retested 8 weeks after the last exposure to the infectious patient. If the second test is positive, LTBI therapy is completed. If it is negative, LTBI therapy is discontinued unless the contact is severely immunocompromised or is younger than 6 months old [40].

5.4 Individuals with Fibrotic Changes on Chest X-Ray

Individuals who have not previously been treated for TB who have fibrotic lesions on chest X-ray indicative of old inactive TB are at high risk of developing TB disease. This is especially true if there are fibrotic lesions greater than 2 cm^2 [42]. These patients are recommended for prioritization of LTBI interventions by most but not all national and international guidelines. To ensure that subclinical TB is not missed and that an alternative diagnosis is not present, after an initial positive test for LTBI these patients should have sputum evaluation and a repeat chest X-ray once sputum cultures are negative to ensure that all radiographic findings remain stable before initiating LTBI treatment.

5.5 Contacts Who Have Previously Been Treated

When an immunocompetent person who has previously completed treatment for TB disease or LTBI is exposed to an infectious TB case, they should undergo a clinical evaluation to rule out TB disease, but generally LTBI treatment is not offered in the United States, as it is believed that they have persistent protective immunity against *M. tuberculosis* that was brought by the past TB infection [43]. If the close contacts who have previously been treated are severely immunocompromised, they are often placed on empiric LTBI treatment after TB disease is excluded.

5.6 Contacts to Drug-Resistant Infectious TB Cases

For a close contact who is diagnosed with LTBI after recent exposure to an infectious MDR TB case, the LTBI regimen is based on the drug susceptibility of the source MDR TB case. If it is susceptible to fluoroquinolones, a fluoroquinolone-based regime is often used because of the significant bactericidal activity of levofloxacin or moxifloxacin and their lower toxicity profile compared to other second-line drugs. Levofloxacin or moxifloxacin may be used alone, or combined with another drug such as ethambutol [37]. Generally, MDR LTBI treatment is

given once daily for 6 months, or possibly for 12 months for those who are severely immunocompromised.

Consider the following questions for treatment options:

1. How likely is it that the individual is newly TB infected? An individual with a documented prior positive test for TB infection is less likely to be newly infected and may not be a candidate for MDR LTBI treatment.
2. How likely is it that the individual is infected with a strain of MDR TB? Assess the Infectiousness of the source patient, the level of the MDR TB exposure (e.g., duration, closeness, and environment), and the contact's risk of exposure to drug-susceptible TB prior to the MDR TB exposure.
3. How likely is it that the individual develops TB disease? A cautious approach to close contacts who are immunocompromised is recommended.

5.7 Pregnancy

The benefits of initiating treatment during pregnancy or within 3 months postpartum should take into account the patient's risk of progression to TB disease. While guidelines differ, there is general agreement that close contacts should initiate LTBI treatment immediately. 3HP is not recommended in pregnancy; otherwise, treatment considerations are the same as for nonpregnant patients. If INH is used, pyridoxine should be co-administered to prevent peripheral neuropathy.

For pregnant PLHIV, there is less consensus. The WHO recommends LTBI treatment in all pregnant women with HIV and LTBI [6], while the US Department of Health and Human Services recommends waiting until 3 months after delivery [44]. The USDHS recommendation is based on a trial that was published in 2019 (after the WHO guidelines were published). In this trial, 956 pregnant women with HIV living in high-burden TB settings who had an unknown TST or IGRA status were randomized to receive INH during pregnancy or after 3 months post-partum. The rates of TB incidence and hepatotoxicity were similar between the groups, but the rates of adverse pregnancy outcomes (stillbirth, spontaneous abortion, low birth weight infant, preterm delivery, or congenital anomalies) were higher in those treated with INH during pregnancy [45].

All pregnant women who are not HIV positive and who do not have recent close contact with an infectious TB case should defer LTBI treatment until 3 months postpartum. They require a repeat symptom screen and chest X-ray to rule out the development of TB disease at that time.

5.8 Advanced Age

Although aging itself is not clearly demonstrated to increase the risk of TB reactivation, it is often deemed that the benefit of LTBI treatment among older adults is diminished because of their lower lifetime risk of progression from infection

acquired in the distant past to TB disease. Furthermore, age is associated with an increased risk of INH-induced hepatitis. LTBI treatment among older adults has not been offered unless there is a high risk of progression to TB (e.g., recent TB exposure in an immunocompromised host). However, there is an emerging interest in addressing selected subgroups of older adults. A recent large-scale study on daily rifampin for 4 months as a LTBI treatment regimen showed that age was not associated with adverse events due to rifampin [46]. As the mortality and other clinical outcomes of older adults with TB disease are worse than younger patients [47], it is suggested that the risk-benefit ratio of the use of safer rifamycin-based regimens for LTBI be reconsidered in a selected older population [48].

5.9 Low Suspicion for Active TB Disease

Two months of rifampin and pyrazinamide daily is an effective treatment for LTBI, but is not recommended due to an unacceptable rate of hepatotoxicity. However, if a patient who is initially suspected of having TB disease (but is later determined to have LTBI) completes the initial 2 months of empiric therapy for TB disease with a standard regimen that includes rifampin and pyrazinamide, this is considered adequate therapy for LTBI.

6 Future Directions

The success of LTBI treatment hinges on (1) improved diagnostic tests to accurately predict and identify those who are at risk of progression to TB disease and (2) shorter and safer regimens that they can tolerate with minimal drug–drug interactions. CDC-sponsored TB Trials Consortium is conducting an open-label, multicenter, phase 3 randomized controlled non-inferiority trial that compares the safety and effectiveness of a 6-week regimen of daily rifapentine again the current standard of 12–16 weeks of rifamycin-based treatment for LTBI [49]. In addition, studies on the treatment of MDR LTBI are also underway. Upgraded tools (i.e., diagnostics and treatment regimens) should be combined with interventions to improve the cascade of care in diagnosis and treatment of LTBI to maximize the benefit [50].

References

1. Pai M, Behr MA, Dowdy D, Dheda K, Divangahi M, Boehme CC, et al. Tuberculosis. Nat Rev Dis Primers. 2016;2:16076. https://doi.org/10.1038/nrdp.2016.76.
2. Houben RM, Dodd PJ. The global burden of latent tuberculosis infection: a re-estimation using mathematical modelling. PLoS Med. 2016;13(10):e1002152. https://doi.org/10.1371/journal.pmed.1002152.

3. Sterling TR, Njie G, Zenner D, Cohn DL, Reves R, Ahmed A, et al. Guidelines for the treatment of latent tuberculosis infection: recommendations from the National Tuberculosis Controllers Association and CDC, 2020. MMWR Recomm Rep. 2020;69(1):1–11. https://doi.org/10.15585/mmwr.rr6901a1.
4. WHO. In: Licence: CC BY-NC-SA 3.0 IGO, editor. Global tuberculosis report 2020. Geneva: World Health Organization; 2020.
5. Ghodbane R, Drancourt M. Non-human sources of *Mycobacterium tuberculosis*. Tuberculosis (Edinb). 2013;93(6):589–95. https://doi.org/10.1016/j.tube.2013.09.005.
6. WHO. Latent tuberculosis infection: updated and consolidated guidelines for programmatic management. Geneva: World Health Organization; 2018. Licence: CC BY-NC-SA 3.0 IGO
7. Shea KM, Kammerer JS, Winston CA, Navin TR, Horsburgh CR Jr. Estimated rate of reactivation of latent tuberculosis infection in the United States, overall and by population subgroup. Am J Epidemiol. 2014;179(2):216–25. https://doi.org/10.1093/aje/kwt246.
8. Churchyard GJ, Fielding KL, Lewis JJ, Coetzee L, Corbett EL, Godfrey-Faussett P, et al. A trial of mass isoniazid preventive therapy for tuberculosis control. N Engl J Med. 2014;370(4):301–10. https://doi.org/10.1056/NEJMoa1214289.
9. Getahun H, Matteelli A, Chaisson RE, Raviglione M. Latent *Mycobacterium tuberculosis* infection. N Engl J Med. 2015;372(22):2127–35. https://doi.org/10.1056/NEJMra1405427.
10. Comstock GW, Livesay VT, Woolpert SF. The prognosis of a positive tuberculin reaction in childhood and adolescence. Am J Epidemiol. 1974;99(2):131–8. https://doi.org/10.1093/oxfordjournals.aje.a121593.
11. Sutherland I. Recent studies in the epidemiology of tuberculosis, based on the risk of being infected with tubercle bacilli. Adv Tuberc Res. 1976;19:1–63.
12. Zak DE, Penn-Nicholson A, Scriba TJ, Thompson E, Suliman S, Amon LM, et al. A blood RNA signature for tuberculosis disease risk: a prospective cohort study. Lancet. 2016;387(10035):2312–22. https://doi.org/10.1016/s0140-6736(15)01316-1.
13. Horsburgh CR Jr, Rubin EJ. Clinical practice. Latent tuberculosis infection in the United States. N Engl J Med. 2011;364(15):1441–8. https://doi.org/10.1056/NEJMcp1005750.
14. Lewinsohn DM, Leonard MK, LoBue PA, Cohn DL, Daley CL, Desmond E, et al. Official American Thoracic Society/Infectious Diseases Society of America/Centers for Disease Control and Prevention Clinical Practice Guidelines: diagnosis of tuberculosis in adults and children. Clin Infect Dis. 2017;64(2):111–5. https://doi.org/10.1093/cid/ciw778.
15. Menzies D, Wong T, editors. Chapter 6. Treatment of latent tuberculosis infection. In: Canadian tuberculosis standards. 7th ed. Canadian Thoracic Society, Canadian Lung Association, Public Health Agency of Canada; 2013.
16. Menzies D, Gardiner G, Farhat M, Greenaway C, Pai M. Thinking in three dimensions: a web-based algorithm to aid the interpretation of tuberculin skin test results. Int J Tuberc Lung Dis. 2008;12(5):498–505.
17. Nachiappan AC, Rahbar K, Shi X, Guy ES, Mortani Barbosa EJ Jr, Shroff GS, et al. Pulmonary tuberculosis: role of radiology in diagnosis and management. Radiographics. 2017;37(1):52–72. https://doi.org/10.1148/rg.2017160032.
18. Mtei L, Matee M, Herfort O, Bakari M, Horsburgh CR, Waddell R, et al. High rates of clinical and subclinical tuberculosis among HIV-infected ambulatory subjects in Tanzania. Clin Infect Dis. 2005;40(10):1500–7. https://doi.org/10.1086/429825.
19. Hamada Y, Lujan J, Schenkel K, Ford N, Getahun H. Sensitivity and specificity of WHO's recommended four-symptom screening rule for tuberculosis in people living with HIV: a systematic review and meta-analysis. Lancet HIV. 2018;5(9):e515–e23. https://doi.org/10.1016/s2352-3018(18)30137-1.
20. Saukkonen JJ, Cohn DL, Jasmer RM, Schenker S, Jereb JA, Nolan CM, et al. An official ATS statement: hepatotoxicity of antituberculosis therapy. Am J Respir Crit Care Med. 2006;174(8):935–52. https://doi.org/10.1164/rccm.200510-1666ST.
21. Targeted tuberculin testing and treatment of latent tuberculosis infection. American Thoracic Society. MMWR Recomm Rep. 2000;49(Rr-6):1–51.

22. Nahid P, Dorman SE, Alipanah N, Barry PM, Brozek JL, Cattamanchi A, et al. Official American Thoracic Society/Centers for Disease Control and Prevention/Infectious Diseases Society of America Clinical Practice Guidelines: treatment of drug-susceptible tuberculosis. Clin Infect Dis. 2016;63(7):e147–e95. https://doi.org/10.1093/cid/ciw376.
23. Comstock GW. How much isoniazid is needed for prevention of tuberculosis among immunocompetent adults? Int J Tuberc Lung Dis. 1999;3(10):847–50.
24. Ena J, Valls V. Short-course therapy with rifampin plus isoniazid, compared with standard therapy with isoniazid, for latent tuberculosis infection: a meta-analysis. Clin Infect Dis. 2005;40(5):670–6. https://doi.org/10.1086/427802.
25. Menzies D, Dion MJ, Rabinovitch B, Mannix S, Brassard P, Schwartzman K. Treatment completion and costs of a randomized trial of rifampin for 4 months versus isoniazid for 9 months. Am J Respir Crit Care Med. 2004;170(4):445–9. https://doi.org/10.1164/rccm.200404-478OC.
26. Menzies D, Long R, Trajman A, Dion MJ, Yang J, Al Jahdali H, et al. Adverse events with 4 months of rifampin therapy or 9 months of isoniazid therapy for latent tuberculosis infection: a randomized trial. Ann Intern Med. 2008;149(10):689–97. https://doi.org/10.7326/0003-4819-149-10-200811180-00003.
27. Sterling TR, Scott NA, Miro JM, Calvet G, La Rosa A, Infante R, et al. Three months of weekly rifapentine and isoniazid for treatment of Mycobacterium tuberculosis infection in HIV-coinfected persons. AIDS. 2016;30(10):1607–15. https://doi.org/10.1097/qad.0000000000001098.
28. Belknap R, Holland D, Feng PJ, Millet JP, Caylà JA, Martinson NA, et al. Self-administered versus directly observed once-weekly isoniazid and rifapentine treatment of latent tuberculosis infection: a randomized trial. Ann Intern Med. 2017;167(10):689–97. https://doi.org/10.7326/m17-1150.
29. Borisov AS, Bamrah Morris S, Njie GJ, Winston CA, Burton D, Goldberg S, et al. Update of recommendations for use of once-weekly isoniazid-rifapentine regimen to treat latent *Mycobacterium tuberculosis* infection. MMWR Morb Mortal Wkly Rep. 2018;67(25):723–6. https://doi.org/10.15585/mmwr.mm6725a5.
30. Menzies D, Adjobimey M, Ruslami R, Trajman A, Sow O, Kim H, et al. Four months of rifampin or nine months of isoniazid for latent tuberculosis in adults. N Engl J Med. 2018;379(5):440–53. https://doi.org/10.1056/NEJMoa1714283.
31. Villarino ME, Scott NA, Weis SE, Weiner M, Conde MB, Jones B, et al. Treatment for preventing tuberculosis in children and adolescents: a randomized clinical trial of a 3-month, 12-dose regimen of a combination of rifapentine and isoniazid. JAMA Pediatr. 2015;169(3):247–55. https://doi.org/10.1001/jamapediatrics.2014.3158.
32. Diallo T, Adjobimey M, Ruslami R, Trajman A, Sow O, Obeng Baah J, et al. Safety and side effects of rifampin versus isoniazid in children. N Engl J Med. 2018;379(5):454–63. https://doi.org/10.1056/NEJMoa1714284.
33. Martinson NA, Barnes GL, Moulton LH, Msandiwa R, Hausler H, Ram M, et al. New regimens to prevent tuberculosis in adults with HIV infection. N Engl J Med. 2011;365(1):11–20. https://doi.org/10.1056/NEJMoa1005136.
34. Sterling TR, Villarino ME, Borisov AS, Shang N, Gordin F, Bliven-Sizemore E, et al. Three months of rifapentine and isoniazid for latent tuberculosis infection. N Engl J Med. 2011;365(23):2155–66. https://doi.org/10.1056/NEJMoa1104875.
35. Sterling TR, Moro RN, Borisov AS, Phillips E, Shepherd G, Adkinson NF, et al. Flu-like and other systemic drug reactions among persons receiving weekly rifapentine plus isoniazid or daily isoniazid for treatment of latent tuberculosis infection in the PREVENT Tuberculosis Study. Clin Infect Dis. 2015;61(4):527–35. https://doi.org/10.1093/cid/civ323.
36. Forget EJ, Menzies D. Adverse reactions to first-line antituberculosis drugs. Expert Opin Drug Saf. 2006;5(2):231–49. https://doi.org/10.1517/14740338.5.2.231.
37. Curry International Tuberculosis Center and California Department of Public Health. Drug-resistant tuberculosis: a survival guide for clinicians. 3rd ed. 2016.

38. Akolo C, Adetifa I, Shepperd S, Volmink J. Treatment of latent tuberculosis infection in HIV infected persons. Cochrane Database Syst Rev. 2010;2010(1):Cd000171. https://doi.org/10.1002/14651858.CD000171.pub3.
39. Newton SM, Brent AJ, Anderson S, Whittaker E, Kampmann B. Paediatric tuberculosis. Lancet Infect Dis. 2008;8(8):498–510. https://doi.org/10.1016/s1473-3099(08)70182-8.
40. Guidelines for the investigation of contacts of persons with infectious tuberculosis. Recommendations from the National Tuberculosis Controllers Association and CDC. MMWR Recomm Rep. 2005;54(Rr-15):1–47.
41. Lee SW, Oh DK, Lee SH, Kang HY, Lee CT, Yim JJ. Time interval to conversion of interferon-gamma release assay after exposure to tuberculosis. Eur Respir J. 2011;37(6):1447–52. https://doi.org/10.1183/09031936.00089510.
42. Efficacy of various durations of isoniazid preventive therapy for tuberculosis: five years of follow-up in the IUAT trial. International Union Against Tuberculosis Committee on Prophylaxis. Bull World Health Organ. 1982;60(4):555–64.
43. Barnes PF, Cave MD. Molecular epidemiology of tuberculosis. N Engl J Med. 2003;349(12):1149–56. https://doi.org/10.1056/NEJMra021964.
44. Clinicalinfo.hiv.gov. Guidelines for the Prevention and Treatment of Opportunistic Infections in Adults and Adolescents with HIV. https://clinicalinfo.hiv.gov/en/guidelines/adult-and-adolescent-opportunistic-infection/whats-new-guidelines. Accessed 20 May 2021.
45. Gupta A, Montepiedra G, Aaron L, Theron G, McCarthy K, Bradford S, et al. Isoniazid preventive therapy in HIV-infected pregnant and postpartum women. N Engl J Med. 2019;381(14):1333–46. https://doi.org/10.1056/NEJMoa1813060.
46. Campbell JR, Trajman A, Cook VJ, Johnston JC, Adjobimey M, Ruslami R, et al. Adverse events in adults with latent tuberculosis infection receiving daily rifampicin or isoniazid: post-hoc safety analysis of two randomised controlled trials. Lancet Infect Dis. 2020;20(3):318–29. https://doi.org/10.1016/s1473-3099(19)30575-4.
47. Gardner Toren K, Spitters C, Pecha M, Bhattarai S, Horne DJ, Narita M. Tuberculosis in older adults: Seattle and King County, Washington. Clin Infect Dis. 2020;70(6):1202–7. https://doi.org/10.1093/cid/ciz306.
48. Hase I, Gardner Toren K, Hirano H, Sakurai K, Horne DJ, Saito T, Narita M. Pulmonary tuberculosis in older adults: Increased mortality related to tuberculosis within two months of treatment initiation. Drugs Aging. 2021;38(9):807–15. https://doi.org/10.1007/s40266-021-00880-4.
49. ClinicalTrials.gov [Internet]. Bethesda, MD: National Library of Medicine (US). 2000 Feb 29. Identifier: NCT03474029, Assessment of the Safety, Tolerability, and Effectiveness of Rifapentine Given Daily for LTBI (ASTERoiD); 2018 March [cited 2021 Oct 21]; Available from: https://clinicaltrials.gov/ct2/show/NCT03474029
50. Alsdurf H, Hill PC, Matteelli A, Getahun H, Menzies D. The cascade of care in diagnosis and treatment of latent tuberculosis infection: a systematic review and meta-analysis. Lancet Infect Dis. 2016;16(11):1269–78. https://doi.org/10.1016/s1473-3099(16)30216-x.

Part IV
Research Areas

Chapter 11
TB Vaccines: What Type of TB Vaccines Are Studied and Will Be Available in the Future?

Masaji Okada and Yoko Kita

Abstract TB remains a major health threat. It is estimated that 23% of the world's population are infected with *Mycobacterium tuberculosis*, with ten million people falling sick with TB and 1.2 million people dying from TB every year. Bacillus Calmette-Guerin (BCG) has been available for about 100 years; however, it does not provide adequate protection against TB, and it is not an effective treatment for TB.

Further, multidrug-resistant TB is a serious global problem. It is, therefore, necessary to develop novel therapeutic and prophylactic vaccines against TB. To this end, we developed a novel therapeutic vaccine (HVJ-E/HSP65 DNA + IL-12 DNA) against TB. In this chapter, several types of TB vaccines, including novel prophylactic and therapeutic vaccines that have recently been studied will be discussed.

Types of TB vaccines include: (1) protein or adjuvant vaccines, (2) DNA vaccines, (3) viral-vectored vaccines, (4) live recombinant BCG vaccines, and (5) attenuated vaccines.

This review provides a summary of novel vaccines in preclinical stage using mouse, guinea pig, and monkey models. In several promising novel vaccines, the studies were extended to a cynomolgus monkey model, which is currently the best animal model for human TB. This review also presents several recent promising advances in clinical trials on vaccines.

Keywords Prophylactic TB vaccine · Therapeutic TB vaccine · Cytotoxic T cell · Helper T cell · Cynomolgus monkey · Clinical trial

M. Okada (✉) · Y. Kita
Clinical Research Center, National Hospital Organization Kinki-Chuo Chest Medical Center, Sakai, Osaka, Japan
e-mail: okada.masaji.dv@mail.hosp.go.jp

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1 Introduction

TB is a major global threat to health, with about 1.2 million people dying every year from *Mycobacterium tuberculosis* infection. The only TB vaccine currently available is an attenuated strain of *Mycobacterium bovis* Bacillus Calmette-Guerin (BCG) vaccine, and its efficacy against adult TB disease remains controversial. Furthermore, multidrug-resistant TB (MDR-TB) and extremely drug-resistant TB (XDR-TB) are becoming significant global health concerns [1]. Every year, about 400,000 people around the world become infected with MDR-TB, and only a small number of drugs are effective against MDR-TB. Hence, it is necessary to develop therapeutic and prophylactic vaccines against TB. Moreover, developing a vaccine that can prevent reactivation in individuals with latent TB infection (LTBI) may have a significant impact on the global TB burden. Therefore, we developed a novel TB vaccine. Our vaccine is a DNA vaccine that expresses mycobacterial heat shock protein 65 (HSP65) and interleukin-12 (IL-12) delivered by the hemagglutinating virus of Japan (HVJ)-envelope (HVJ-E) [2–6]. In the report by Billeskov et al., our vaccine was one of the therapeutic TB vaccines discussed [5, 7].

This vaccine was found to be more efficient than BCG in a murine TB prophylactic model based on the elimination of *M. tuberculosis* mediated by the induction of cytotoxic T lymphocytes (CTLs) and the production of interferon-gamma (IFN-γ); it has been reported in several studies that CTLs, T helper 1 (Th1) cells, and IFN-γ play key roles in TB vaccines [2–4, 7–16]. Furthermore, in our previous study, we reported that:

1. HVJ-E/pcDNA3.1 HSP65 DNA + IL-12 DNA vaccine showed therapeutic efficacy against MDR-TB in mice.
2. Significant prolongation of survival in XDR-TB-infected mice due to treatment with this vaccine was observed.
3. This vaccine showed therapeutic efficacy in chronic TB disease models that used mice infected with TB in an aerosol chamber [5, 10, 11].

It has been reported that a non-human primate (NHP) model of TB can provide important data for vaccine development [3–5, 17]. In fact, in our previous studies, we evaluated the therapeutic and protective efficacy of the HSP65 DNA + IL-12 DNA vaccine in a cynomolgus monkey model, which is an excellent model for human TB [3–5, 10, 11]. This vaccine showed therapeutic activity in the cynomolgus model in the form of survival prolongation, increased proliferation of peripheral blood lymphocytes (PBLs), and improvement of erythrocyte sedimentation rate (ESR) [5, 10, 11]. It is vitally important to evaluate long-term survival in monkey models as human TB is a chronic infectious disease [4, 5, 10, 11]. Thus, we took advantage of the multiple animal models and accumulated essential data on the vaccine in anticipation of a phase I clinical study [11]. In fact, a phase 1 (investigator initiated) clinical trial of this vaccine has already begun. Based on blood pDNA concentrations, the therapeutic vaccine appears to be safe and well tolerated in patients. Furthermore, TB colony count in the sputum and the Gaffky study

suggested anti-TB efficacy (MDR-TB negative conversion). Anti-TB immunity (IFN-γ and IL-2 production) was augmented in patients who were administered this vaccine.

Thus, the therapeutic and prophylactic efficacy of our DNA vaccine against TB will be discussed in the next section.

(*TB vaccines in clinical trials*) The development of an efficacious TB vaccine strategy relies on a healthy pipeline of TB vaccine candidates that represent a diverse repertoire of formulations and mycobacterial antigens and that induce a broad range of immune responses with different characteristics. Table 11.1 shows eight TB

Table 11.1 TB vaccine candidates

Vaccine	Type	Adjuvant	Vaccine antigens	Induced T cell responses
KCMC-001 [5, 23]	DNA	· HVJ (Hemagglutinating virus of Japan)- envelope · IL-12	HSP65 human TB antigen (HVJ-E/HSP65 DNA+ IL-12 DNA) vaccine	CD8+ T cells CD4+ T cells IFN-γ, IL-2, TNFα
M72/AS01$_E$ [19, 25]	Protein subunit	AS01 adjuvant	Mtb39a Mtb32a	CD4+ T cells CD8+ T cells IFN-γ, IL-2, TNFα
H4:IC31 [20]	Protein subunit	IC31	TB 10.4 (Rv0288) Ag85B	CD4+ T cells IFN-γ, IL-2, TNFα
H56:IC31 [21]	Protein subunit	IC31	Ag85B ESAT6 (Rv3875) Rv2660E	CD4+ T cells IFN-γ, IL-2, TNFα
ID93 + GLA-SE [22]	Protein subunit	GLA-SE (TLR4 agonist)	Rv1813 Rv2608 Rv3619 Rv3620	CD4+ T cells IFN-γ, IL-2, TNFα
MVA85A [27]	Viral vector (MVA: Modified vaccinia virus Ankara)		Ag85A (Rv3804c)	CD4+ T cells IFN-γ, IL-2, TNFα
AERAS-402	Viral vector (adenovirus)		Ag85A Ag85B TB10.4	
VPM1002 [29]	Recombinant BCG		Listeriolysin gene Delete urease C gene	CD4+ T cells CD8+ T cells

vaccine candidates including a recombinant BCG (rBCG) vaccine. They are currently in clinical testing for prophylactic, post-exposure, or therapeutic indications.

1. *Adjuvanted protein subunit vaccines*: Subunit vaccines are based on protein antigens administered with adjuvants. These are primarily developed as prophylactic or post-exposure (therapeutic) vaccines that boost immune responses that were initially primed by BCG or TB infection for prevention of TB infection, active TB, or recurrent disease. Subunit vaccines that are currently in clinical testing include M72/AS01$_E$, H4:IC31, H56:IC31, and ID93 + GLA-SE [18]. Some of these vaccines are also tested as therapeutic vaccines (for example, H56:IC31 and ID93 + GLA-SE) to prevent recurrence in patients who have completed chemotherapy for active TB (Table 11.1).
2. *DNA vaccines*: KCMC-001 (HSP65 + IL-12 DNA vaccine), which is a DNA vaccine, induces intracellular production of HSP65 antigen in vivo and activates T cell systems (Table 11.1) [2, 4, 5].
3. *Viral-vectored vaccines*: Live attenuated non-replicating viruses can be engineered to deliver genes encoding the antigens of interest into host cells. Such vaccines allow for the intracellular production of the antigen in vivo and activate cells of the immune system and therefore do not need to be adjuvanted. Viral-vectored vaccines are developed as both prophylactic and post-exposure vaccines that boost responses primed by BCG or TBinfection. Two viral-vectored TB vaccine candidates, MVA85A and Ad5Ag85A, are currently in clinical testing as prime-boost combinations, including trials of the MVA85A candidate administered as an aerosol into the airways [18].
4. (a) Whole-cell live vaccines: Two of these vaccines, VPM1002 (a rBCG vaccine) and MTBVAC (a live attenuated Mtb vaccine), are currently in clinical trials. VPM1002 is also being assessed as a post-exposure vaccine for the prevention of active TB recurrence (Table 11.1).

   (b) Whole-cell inactivated vaccines: Based on the classical paradigm of vaccine development, these products include RUTI, Mycobacterium vaccae-based vaccines, and the *Mycobacterium obuense*-based DAR-901 vaccine [18].

Promising clinical trials of several novel vaccines, including the M72/AS01$_E$, H4:IC31, H56:IC31, VPM1002, and ID93 vaccines, will be discussed [19–22].

2 Prophylactic DNA Vaccines

2.1 The HVJ-Liposome/HSP65 DNA + IL-12 DNA Vaccine

The immunogenicity and protective efficacy of DNA vaccine combinations expressing mycobacterial HSP65 and IL-12 using gene gun bombardment and the HVJ-liposome method were investigated [2]. A mouse IL-12 expression vector (mIL-12

DNA) encoding single-chain IL-12 proteins consists of p40 and p35 subunits was constructed. In a mouse model, a single gene gun vaccination with the combination of HSP65 DNA and mIL-12 DNA provided a remarkably high degree of protection against challenges with virulent *M. tuberculosis*; bacterial numbers in the lungs of these mice were 100 times lower than those in BCG-vaccinated mice. To explore the clinical use of the DNA vaccines, we evaluated HVJ-liposome encapsulated HSP65 DNA and mIL-12 DNA (HSP65 + mIL-12/HVJ). Compared to gene gun vaccination, the HVJ-liposome method improved the protective efficacy of the HSP65 DNA vaccine. HSP65 + mIL-12/HVJ induced CD8+ CTL activity against HSP65 antigen. Most importantly, HSP65 + mIL-12/HVJ vaccination resulted in a greater degree of protection than that evoked by BCG. This protective efficacy was associated with the emergence of IFN-γ-secreting T cells, activation of proliferative T cells, and cytokine (IFN-γ and IL-2) production upon stimulation with HSP65 and antigens from *M. tuberculosis*. These results suggest that HSP65 + IL-12/HVJ may be a promising new vaccine candidate that is superior to the BCG vaccine [2].

2.2 The HVJ-E/HSP65 DNA + IL-12 DNA Vaccine

We further proceeded in our efforts to develop a novel vaccine with the use of HVJ-E [8].

A novel TB vaccine expressing HSP65 and IL-12 delivered by HVJ-E was evaluated in mice with *M. tuberculosis* infection. Bacterial load reductions and histopathological assessments were used to determine efficacy.

Vaccination using BCG prime with HVJ-E/HSP65 + IL-12 DNA boost resulted in significant protective efficacy against *M. tuberculosis* infection in the lungs of mice (> 10,000 times greater than BCG alone; Fig. 11.1a) [9]. In addition to bacterial load reduction, histopathological analysis of the lungs revealed significant protective efficacy. Furthermore, the vaccine increased the number of IFN-γ-secreting T cells. In conclusion, this vaccine showed extremely significant protection against TB in a mouse model, and this finding is consistent with the results of a similar study in a cynomolgus monkey model. The results suggested that further development of the vaccine for eventual testing in clinical trials may be warranted [9].

The in vivo necessity of CD8+ and CD4+ T cells for the prophylactic efficacy of this vaccine was demonstrated in mice [5]. Treatment with anti-CD8 antibody alone or anti-CD4 antibody alone during the whole immunization period induced an increase in the number of TB in vaccinated mice. Anti-CD8 antibody treatment and anti-CD4 antibody treatment synergistically increased the number of TB (Fig. 11.1b) [10].

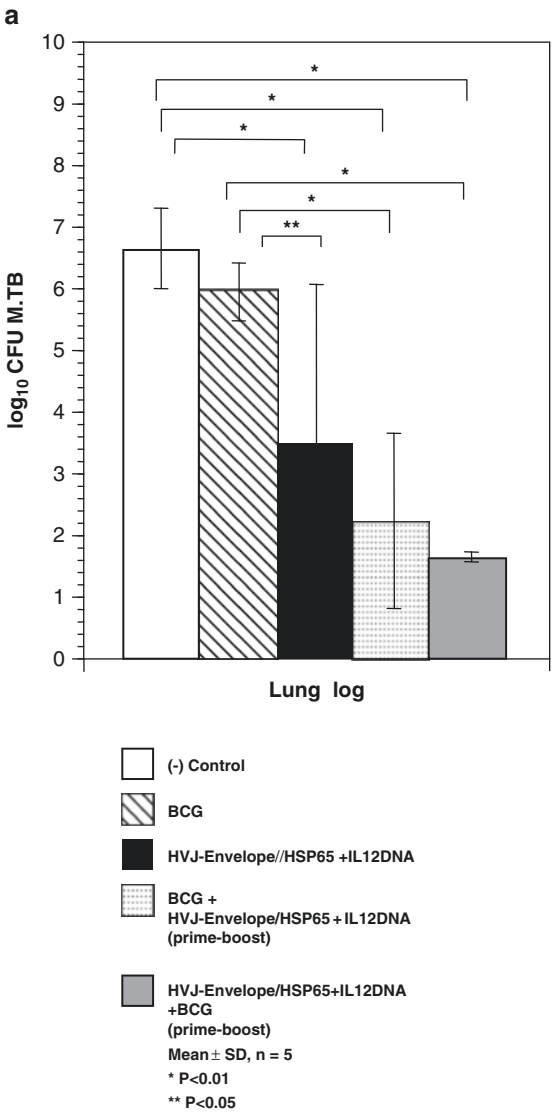

Fig. 11.1 (**a**) Mouse protection studies using the prime-boost method. Groups of mice vaccinated with HVJ-envelope (HVJ-E) DNA and/or BCG were challenged with intravenous injection of *M. tuberculosis* H37Rv [9]. (**b**) Vaccine (HVJ-E/HSP65 DNA+ IL-12 DNA vaccine) induces differentiation of cytotoxic T cells and type 1 helper T cells. (**c**) Therapeutic efficacy of HVJ-E/HSP65 DNA+ IL-12 DNA vaccine on MDR-TB infection in TNFR gene-disrupted DBA/1 mice [5]

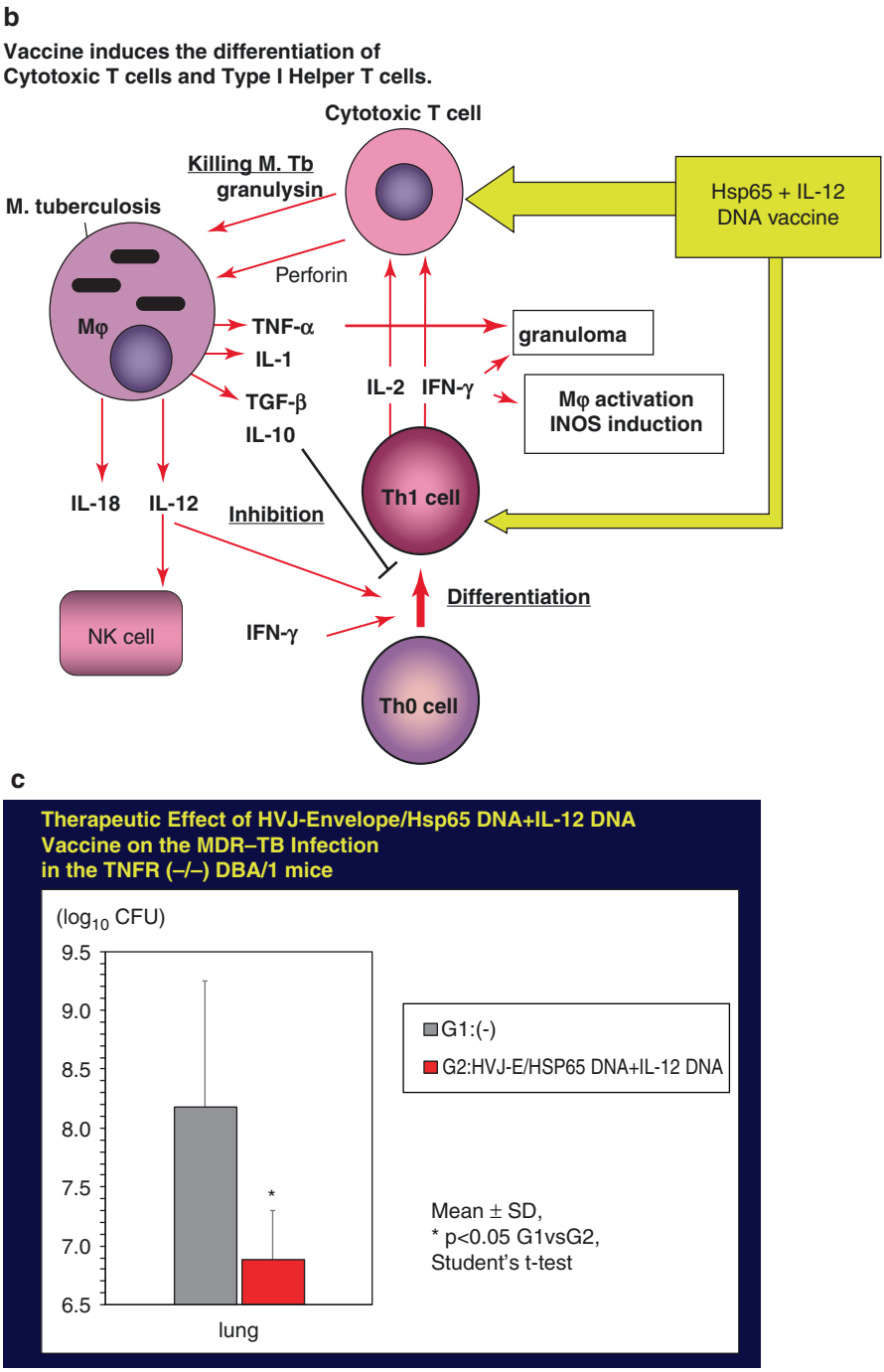

Fig. 11.1 (continued)

3 Prophylactic Vaccine (NHP Model)

We developed a novel TB vaccine that is a combination of the DNA vaccines expressing HSP65 and human IL-12 delivered by HVJ-liposome (HSP65 + IL-12/HVJ) [3]. Compared to the BCG vaccine, this vaccine provided remarkable protective efficacy in mouse and guinea pig models based on the induction of CTL activity and the improvement of histopathological TB lesions, respectively. Furthermore, we extended our studies to a cynomolgus monkey model, which is currently the best animal model for human TB [3]. Based on an assessment of mortality, ESR, body weight, chest X-ray findings, and immune responses in an NHP model, this novel vaccine has a higher level of protective efficacy than BCG. Furthermore, a synergistic effect was observed in TB-infected cynomolgus monkeys (100% survival) with the combination of HSP65 + IL-12/HVJ and BCG using the priming-booster method. These data indicate that our novel DNA vaccine may be useful against *M. tuberculosis* in human clinical trials (Fig. 11.2a) [3].

To evaluate the TB vaccine we developed, we used an NHP model infected with *M. tuberculosis*. The experiment involved 32 monkeys, and the results showed that the protective efficacy of HSP65 + IL-12/HVJ and BCG using the priming-booster method was very strong in TB-infected cynomolgus monkeys [3]. All four monkeys (100%) from the group of BCG priming and DNA vaccine (HVJ-liposome/HSP65 + IL-12 DNA vaccine) booster were alive for more than 12 months after infection (Fig. 11.2a). In contrast, only two of the six monkeys in the BCG Tokyo group were alive (33% survival) [3]. Half the monkeys in the saline (control) group and the DNA vaccine-priming and BCG Tokyo vaccine booster group were alive for more than 12 months. In addition, HSP65 + hIL-12/HVJ improved ESR and chest X-ray findings. Production of IL-2 and IFN-γ and proliferation of PBL were augmented in the group vaccinated with HSP65 + hIL-12/HVJ (data not shown). Taken together, these results clearly demonstrate that BCG priming and HSP65 + hIL-12/HVJ booster can have extremely strong protective efficacy against *M. tuberculosis* in a cynomolgus monkey model.

Furthermore, survival prolongation was observed in monkeys that were administered the combination of BCG and a DNA vaccine (HVJ-E/HSP65 DNA + IL-12 DNA vaccine) even when the boost was performed a long time (4 months) after the prime [13]. Figure 11.2b shows the human IL-12 expression vector (human IL-12 DNA) encoding single-chain IL-12 proteins composed of p40 and p35 subunits was constructed. HSP65DNA + human IL-12 DNA was constructed for preclinical (monkey) and clinical vaccine that contains two kinds of DNA in one plasmid vector (pVAX1) (Fig.11.2b). This combination also improved ESR, increased body weight, and augmented the proliferation of PBLs and the production of IL-2 at higher levels than BCG alone or saline [13].

With the long-term prime-boost method and a vector containing two kinds of genes in one plasmid, the most reproducible and prophylactic efficacy based on survival prolongation was observed in monkeys (BCG prime-DNA vaccine boost). The combination of BCG prime and DNA vaccine boost improved survival (100%

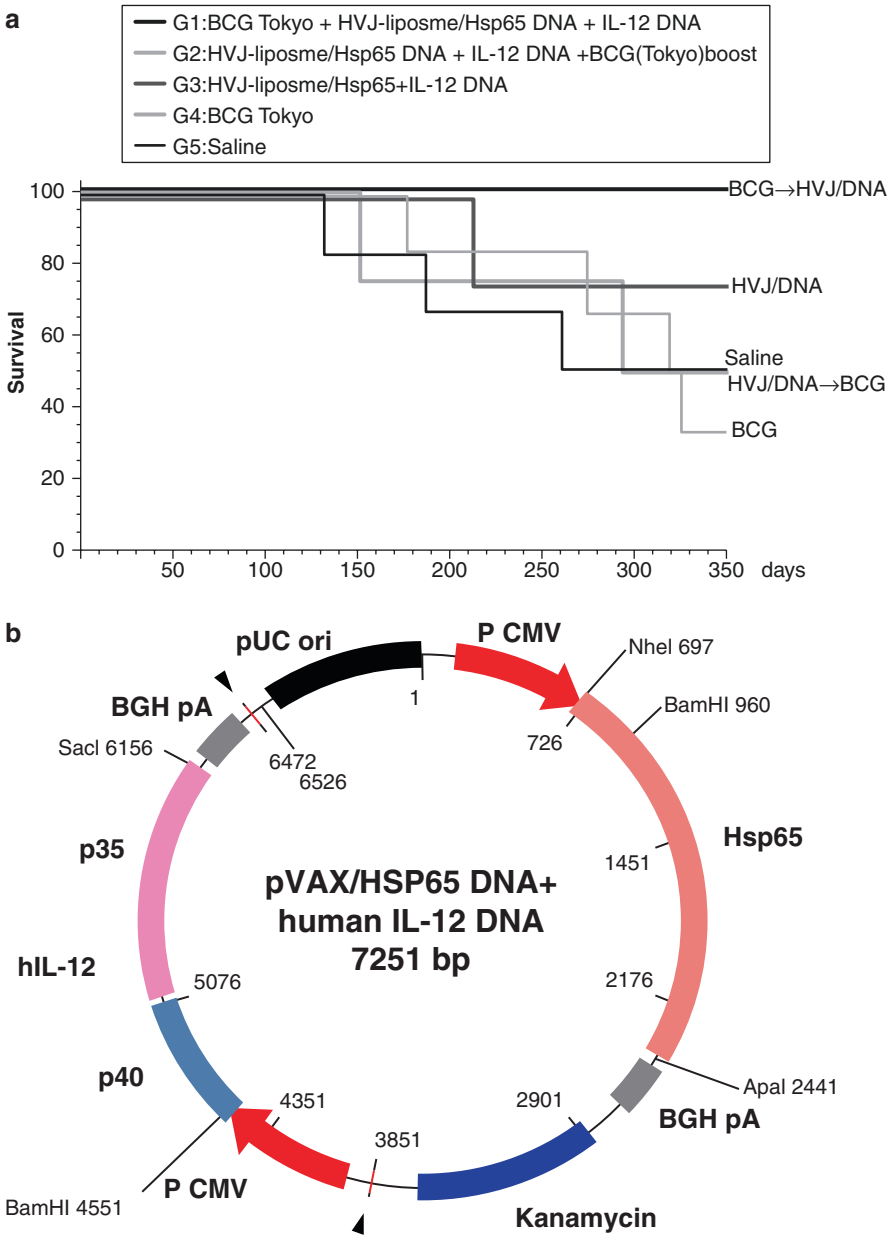

Fig. 11.2 (**a**) Prophylactic efficacy of HVJ-liposome/HSP65 DNA+ human IL-12 DNA vaccine against TB in cynomolgus monkeys [3]. (**b**) Construction of DNA vaccine for preclinical and clinical trials. HVJ-E/HSP65 DNA+ human IL-12 DNA vaccine was constructed for preclinical and clinical vaccine that contains two kinds of DNA in one plasmid vector (pVAX1). (**c**) Therapeutic efficacy of HVJ-E/HSP65 DNA+ human IL-12 DNA vaccine against TB in cynomolgus monkeys [5]

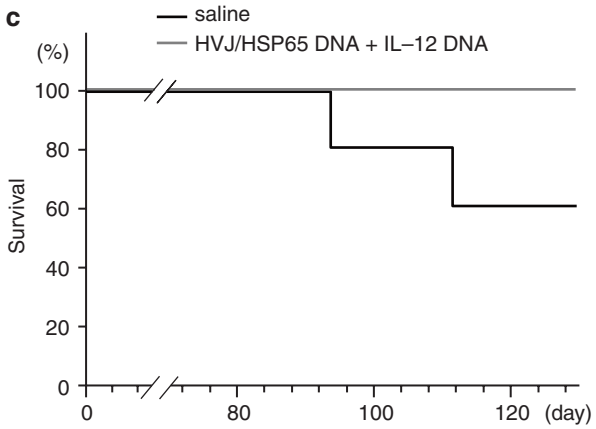

Fig. 11.2 (continued)

and 80% at 230 days and 360 days after TB challenge, respectively). In contrast, survival of monkeys in the BCG vaccine alone group was 40% at 360 days. Thus, in an experimental model with a long interval (4 months) between the prime and the boost, we observed the prophylactic efficacy of the BCG prime and DNA vaccine boost.

4 Therapeutic DNA Vaccine

4.1 Therapeutic Vaccine (Mouse)

We developed a novel TB vaccine (HVJ-E/HSP65 DNA + IL-12 DNA) that has therapeutic efficacy and remarkable protective efficacy via CD8+ and CD4+ T cells in murine models compared to the saline controls; this is based on the number of colony-forming units (CFUs) of MDR-TB and the survival of XDR-TB-challenged mice as shown in Fig. 11.1c [5].

All Balb/c mice in the control group died of TB within 160 days after XDR-TB infection. In contrast, mice treated with this vaccine had significantly prolonged survival periods as shown by statistical analysis ($P < 0.05$). These findings show that this vaccine has therapeutic activity against XDR-TB. After intravenous challenge with MDR-TB, CFUs in the lungs, spleen, and liver were counted and the therapeutic efficacy of the vaccine was evaluated. As shown in Fig. 11.1c, treatment with this DNA vaccine significantly reduced bacterial load in mice with MDR-TB infection compared to the saline (control) group ($P < 0.05$) [5].

4.2 Therapeutic Vaccine (NHP)

We extended our studies to a cynomolgus monkey model, which is currently the best animal model for human TB. This vaccine showed therapeutic efficacy (survival and immune responses) in the TB-infected monkeys. The therapeutic activity of this vaccine was evaluated in an NHP model infected with *M. tuberculosis* [5]. The immune responses of cynomolgus monkeys 11 weeks after challenge with the Erdman strain of *M. tuberculosis* (5×10^2) via intratracheal instillation were studied. In monkeys of the therapeutic vaccination group, the proliferation of PBLs was augmented following administration of this vaccine. Within 19 weeks after the TB challenge, this vaccine also improved the survival of monkeys in the vaccination group compared to monkeys in the saline (control) group (Fig. 11.2c).

This vaccine showed significant therapeutic activity against TB as indicated by:

1. Prolonged survival of mice infected with XDR-TB.
2. Decrease in TB CFUs in the lungs, liver, and spleen of mice infected with MDR-TB and drug-sensitive TB (H37RV).
3. Decrease in TB CFUs in the lungs, liver, and spleen of mice challenged with TB in the in vivo humanized immune model of SCID-PBL/hu.
4. Augmentation of immune responses in a cynomolgus monkey model, which closely mimics human TB disease.

It is important to evaluate the survival of the monkeys [3]. Within 19 weeks after the TB challenge, we observed an increase in the survival rate of monkeys treated with this vaccine compared to monkeys in the control group who were treated with saline. In a mouse model, this vaccine showed therapeutic activity even against XDR-TB, which is resistant to RFP, INH, SM, EB, KM, EVM, TH, PAS, LEFX, and PZA and is only sensitive to CS. Thus, our results with this vaccine in the murine and cynomolgus monkey therapeutic models should provide a strong reason to move this vaccine into clinical trials. Furthermore, we set up a mouse model of chronic TB disease where mice infected with TB in an aerosol chamber were assessed [9]. In this model, we also observed the therapeutic efficacy of this vaccine. Thus, we took advantage of multiple animal models to accumulate essential data on the HVJ-E DNA vaccine in anticipation of a phase I clinical trial [5, 23].

4.3 Preclinical Trial (Toxicology·Safety Pharmacology)

Furthermore, in these experimental conditions, safety pharmacology study and toxicology test of preclinical study for human clinical trial were conducted using monkeys that were administered GMP-level DNA vaccines [23].

1. Single-dose toxicity test was performed by examining monkeys that were subcutaneously administered a high dose of this vaccine. Toxicity was evaluated by assessing the general state, food consumption level, body weight, blood examination, and blood biochemistry.
2. Repeated-dose toxicity test of GLP levels was planned with HVJ-E/pVAX-HSP65 DNA + hIL-12 DNA vaccine (GMP level) for clinical trials. The vaccine was repeatedly administered intramuscularly to cynomolgus monkeys. It was planned such that the repeated-dose toxicity test would include evaluation tests for acute toxicity, local irritability, toxicokinetics (TK), and the central nervous system at the GLP level.
3. TK was studied by analyzing the concentration of human IL-12 in the blood of monkeys vaccinated with HVJ-E/pVAX-HSP65 DNA + hIL-12 DNA vaccine (GMP level) for clinical trials. ELISA will be used to evaluate human IL-12.
4. Safety pharmacology study of GLP levels was planned to include the cardiovascular system, respiratory system, and body temperature. The blood pressure, heart rate, electrocardiographic data, respiratory function (respiratory rate and ventilation volume), and body temperature of the vaccinated monkeys were evaluated.

4.4 Clinical Trial (Phase I)

The plan for clinical trial of this vaccine on patients with MDR-TB (resistant to RFP and INH) is shown in Fig. 11.3. The principal evaluations in the clinical trial were safety and approval, and the secondary evaluations were anti-TB efficacy (decrease in the number of TB in the sputum of patients with MDR-TB) and immune responses against *M. tuberculosis*. The trial will be conducted in hospitals of the National Hospital Organization in Japan.

Phase I Investigator-initiated clinical trial

The phase 1 clinical trial has already begun, and the subjects are human patients with MDR-TB. These patients received intramuscular administrations of 909 μg pDNA +909 m NAU HVJ-E (HSP65 DNA + IL-12 DNA) three times. The primary evaluations were safety and tolerability, and the secondary evaluation was anti-TB efficacy (sputum culture conversion). The safety and tolerability of this therapeutic vaccine to patients are evaluated based on the blood pDNA concentration. Furthermore, anti-TB efficacy (MDR-TB negative conversion) was suggested by the Gaffky study and colony count of TB in the sputum. Anti-TB immunity (IFN-γ and IL-2 production) was augmented in vaccinated patients between 14 and 126 days.

In conclusion, these data indicate that the HSP65 DNA + IL-12 DNA vaccine is useful against XDR-TB and MDR-TB through T cells as a treatment for humans in clinical settings.

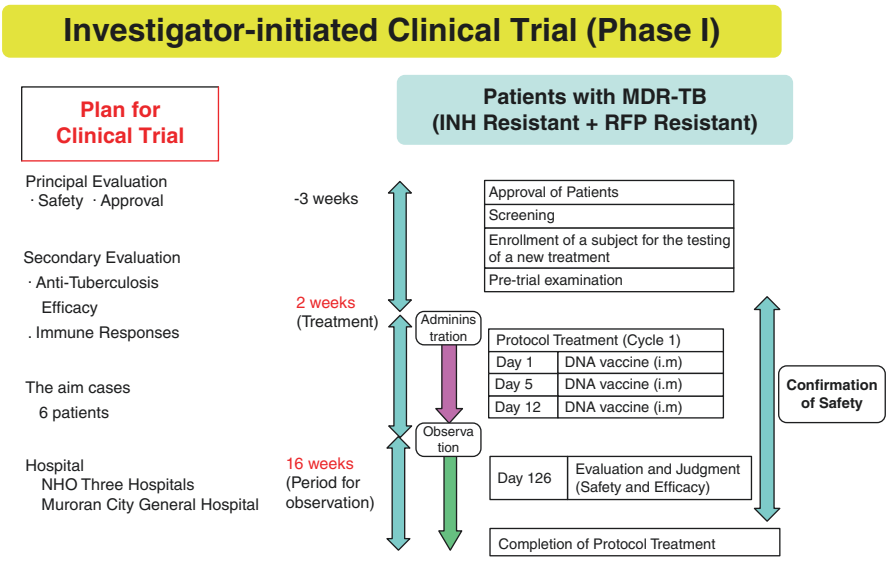

Fig. 11.3 Investigator-initiated (phase I) clinical trial plan of KCMC-001 vaccine (HVJ-E/HSP65 DNA+ IL-12 DNA vaccine)

5 Clinical Trial of Novel Vaccines

5.1 Protein or Adjuvant TB Vaccine

5.1.1 Final Analysis of a Trial of M72/AS01$_E$ Vaccine for TB Prevention

The M72 vaccine is a fusion molecule consisting of two antigens that are strong targets for Th1 cells in PPD-positive individuals. Rv1196 (MTB32) is inserted into the middle of the serine protease Rv0125 (MTB39), which is thus present as two fragments. Two phase I trials of the M72 vaccine on healthy PPD-negative adults were completed in the USA and Belgium. Reed et al. reported that the vaccine is safe, well tolerated, and could induce antigen-specific humoral and cell-mediated immune responses [24].

According to Van Der Meeren et al., in phase II clinical trial, results of an earlier analysis of a trial of the M72/AS01$_E$ candidate vaccine against *M. tuberculosis* showed that the vaccine afforded 54.0% protection against active pulmonary TB disease to infected adults without evident safety concerns [25]. Tait et al. reported the results of the 3-year final analysis of efficacy, safety, and immunogenicity (Fig. 11.4) [19].

They enrolled adults 18–50 years of age with *M. tuberculosis* infection (defined as positive results on IFN-γ release assay [IGRA]) without evidence of active TB

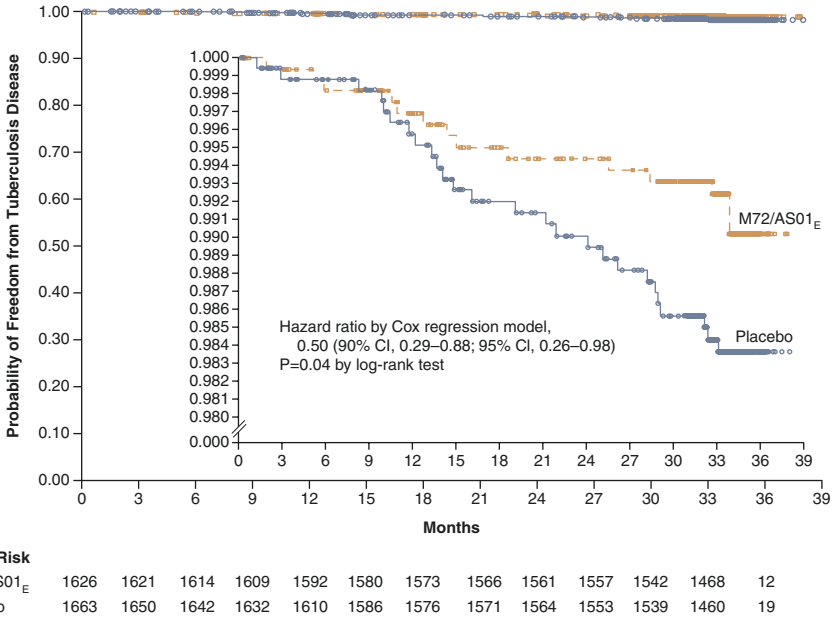

Fig. 11.4 Final analysis of M72/AS01$_E$ vaccine for TB prevention in phase IIb clinical trial [19]. *Kaplan–Meier estimate of definite pulmonary TB according to the first-case definition.* Figure 11.4 shows the Kaplan–Meier estimate of the probability of freedom from TB disease according to the first-case definition (i.e., bacteriologically confirmed pulmonary TB not associated with HIV infection and diagnosed before the initiation of treatment for TB). The analysis was conducted in the according-to-protocol efficacy cohort (3289 participants: 1626 in the M72/AS01$_E$ group and 1663 in the placebo group). The time shown is the time from the beginning of follow-up (i.e., 30 days after dose 2). The inset shows the same data on an enlarged y-axis (with permission from N Engl J Med; [19])

disease at centers in Kenya, South Africa, and Zambia. Participants were randomly assigned on a 1:1 ratio to receive two doses of either M72/AS01$_E$ or placebo administered 1 month apart. The primary objective was to evaluate the efficacy of M72/AS01$_E$ in the prevention of active pulmonary TB disease according to the first-case definition (i.e., bacteriologically confirmed pulmonary TB not associated with human immunodeficiency virus infection). Participants were followed for 3 years after the second dose. Participants with clinical suspicion of TB provided sputum samples for polymerase chain reaction assay, mycobacterial culture, or both. Humoral and cell-mediated immune responses were evaluated until month 36. Safety was assessed for all participants.

A total of 3575 participants underwent randomization. Of these, 3573 received at least one dose of M72/AS01$_E$ or placebo, and 3330 received both planned doses. Of the 3289 participants in the according-to-protocol efficacy cohort, 13 of the 1626 participants in the M72/AS01$_E$ group, compared to 26 of the 1663 participants in the placebo group, had TB that satisfied the first-case definition (incidence: 0.3 versus 0.6 cases per 100 person-years). The vaccine efficacy at month 36 was 49.7%

(Fig. 11.4). In the M72/AS01$_E$ group, the concentrations of M72-specific antibodies and the number of M72-specific CD4+ T cells increased after the first dose and were sustained throughout the follow-up period. Serious adverse events, potential immune-mediated diseases, and deaths occurred at similar frequencies in the two groups.

In conclusion, vaccination with M72/AS01$_E$ elicited an immune response in adults infected with *M. tuberculosis* and provided protection against progression to pulmonary TB disease for at least 3 years (Table 11.1).

Many TB vaccine candidates are in preclinical and clinical development; however, due to limited global resources, only a few can advance to large-scale efficacy trials.

The antigen-specific T cell responses induced by six novel TB vaccine candidates (M72/AS01$_E$, MVA85A, AERAS-402, H1:IC31, H56:IC31, and ID93 + GLA-SE) have been compared.

In M.tb-uninfected and M.tb-infected individuals, M72/AS01$_E$ induced greater memory Th1 cytokine expressing CD4 T cell response than the other novel vaccine candidates [26].

Since M72/AS01$_E$ induced the greatest memory CD4 T cell response, it can be said to demonstrate the best vaccine take.

5.1.2 H4:IC31 Protein Vaccine

H4:IC31 is a candidate subunit vaccine that consists of a recombinant fusion protein (H4) and an IC31 adjuvant that signals through the toll-like receptor 9 and contains the mycobacterial antigens Ag85 and TB10.4, which do not cross-react with QuantiFERON-TB Gold In-tube assay (QFT) antigens [20].

In the phase II trial, 990 adolescents in a high-risk setting who had undergone neonatal BCG vaccination were randomly assigned to receive the H4:IC31 vaccine, BCG revaccination, or placebo [20]. With QFT, all the participants tested negative for *M. tuberculosis* infection. The primary outcomes were safety and *M. tuberculosis* infection, defined as initial conversion on QFT that was performed every 6 months during a 2-year period. The secondary outcomes were immunogenicity and sustained QFT conversion to a positive test.

The BCG and H4:IC31 vaccines were immunogenic. QFT conversion occurred in 44 of 308 participants (14.3%) in the H4:IC31 group, in 41 of 312 participants (13.1%) in the BCG group, and in 49 of 310 participants (15.8%) in the placebo group. The rate of sustained conversion was 8.1% in the H4:IC31 group, 6.7% in the BCG group, and 11.6% in the placebo group. Neither the H4:IC31 vaccine nor the BCG vaccine prevented initial QFT conversion. However, the BCG vaccine reduced the rate of sustained QFT conversion, with an efficacy of 45.4% (P = 0.03), whereas the efficacy of the H4:IC31 vaccine was 30.5% (P = 0.16). These findings may inform clinical development of new vaccine candidates (Table 11.1).

5.1.3 H56:IC31

Suliman et al. sought to define the optimal dose and schedule of H56:IC31, which is an experimental TB vaccine consisting of Ag85B, ESAT-6, and Rv2660c, for M.tb-infected and M.tb-uninfected adults [21]. They enrolled 98 healthy HIV-uninfected BCG-vaccinated South African adults. M.tb infection was defined based on QFT. QFT-negative participants received two vaccinations with different concentrations of H56 in 500 nmol of IC31 to enable dose selection for further vaccine development. Subsequently, QFT-positive and QFT-negative participants were randomized to receive two or three vaccinations to compare potential schedules. Participants were followed for safety and immunogenicity for 292 days. It was found that H56:IC31 showed acceptable reactogenicity profiles regardless of vaccination frequency, dose, or M.tb infection. No serious vaccine-related adverse events were observed [21].

In conclusion, two or three H56:IC31 vaccinations of the lowest dose induced durable antigen-specific CD4 T cell responses with acceptable safety and tolerability profiles in M.tb-infected and M.tb-uninfected adults.

6 Clinical Trial of Novel Vaccines

6.1 Viral-Vectored Vaccines

6.1.1 MVA85A

MVA85A is a modified vaccinia virus Ankara (MVA) strain expressing antigen 85A, another member of the Ag85 family of protective antigens [27]. In phase I studies in humans, MVA85A was found to be safe and well tolerated, and this vaccine induced strong immune responses, particularly in previously BCG-vaccinated individuals. Boosting BCG vaccination with MVA85A downregulates the immunoregulatory cytokine TGFβ1. MVA85A-induced cellular immune responses in UK volunteers. The safety and immunogenicity of MVA85A in West Africans are the rationale for its accelerated development as a booster vaccine for TB. CD4+ T cells responses were predominantly stimulated, and CD8+ T cell responses were observed in subjects with HLA B-35.

In the study by Tameris et al., 2797 infants (1399 infants allocated to MVA85A, and 1398 infants allocated to placebo) were enrolled. MVA85A was well tolerated, and it induced modest cell-mediated immune responses (Table 11.1). However, MVA85A was found to have no efficacy against TB or *M. tuberculosis* infection in infants [27].

AERAS-402 DNA. This DNA vaccine is intended for use as a boosting vaccine in BCG-primed individuals. The vaccine is a serotype 35 adenovirus that is

incapable of replicating, and it contains DNA that expresses a fusion protein created from three *M. tuberculosis* antigens: 85A, 85B, and TB 10.4.

Promising data on the prevention of disease in NHPs using a viral-vectored TB vaccine candidate was reported**.**

A TB vaccine candidate based on a CMV vector that expresses 6 or 9 *Mtb* antigens was designed [28]. This vector was tested in rhesus macaques and was shown to afford profound protection against TB disease [28]. As expected, the vaccine induced profound CD4 and CD8 T cell responses and marked IFN-γ and TNF secretion. Despite some disadvantages of CMV-vectored vaccines, the CMV-based TB vaccine is a promising candidate. A new vaccine that offers no added value to BCG-immunized individuals will face major issues before it can be further developed. Further, a recent study revealed that boosting BCG with M72 or H56 vaccines does not enhance BCG-induced protection in NHPs.

7 Clinical Trials of Novel Vaccines

7.1 Live rBCG Vaccines

7.1.1 VPM1002 [29]

This is one of the most advanced TB vaccines, and Kaufmann et al. improved it by genetic modification [29]. It is an rBCG that expresses listeriolysin from *Listeria monocytogenes* and is devoid of urease C. Development of this vaccine began in the 1990s with the aim of improving BCG by giving it the capacity to stimulate a broader and more efficacious T cell response (Table 11.1).

VPM1002 has successfully completed phase I and phase IIa clinical trials, and this proves its safety and immunogenicity in adults and neonates. A phase II clinical trial on HIV-exposed and HIV-unexposed neonates has been completed and awaits unblinding. A phase III clinical trial on HIV-exposed and HIV-unexposed neonates is being prepared and is expected to start in 2020.

8 Clinical Trial of Novel Vaccine

8.1 TB DNA Vaccines

We discussed the HSP65 DNA + IL-12 DNA vaccine, a DNA vaccine, in sections 2.1–2.5.

8.1.1 BCG Revaccination

There have been recent findings regarding the canonical BCG vaccine.

Two recent studies on BCG immunization reported a significant impact of vaccination regimens [20, 30]. In one of the studies, NHPs were intravenously immunized with BCG [30]. The study results showed that the protection induced in NHPs following intravenous immunization with BCG is more profound than that induced following intradermal or aerogenic BCG vaccination [30].

The other study tested the outcome of BCG booster vaccination in *Mtb*-unexposed adults [20]. Nemes et al. reported the results of a phase IIb trial on the prevention of Mtb infection and showed that BCG revaccination affords significant protection against sustained IGRA conversion in South African adolescents who received BCG at birth.

These two studies provide strong evidence that the outcome of BCG vaccination is significantly influenced by the mode of administration, notably the route of immunization (intravenous) and vaccination schedule (pre-exposure revaccination). In conclusion, the BCG vaccine still has room for improvement.

9 Therapeutic Vaccine and Preventive Vaccine

9.1 ID93 + GLA-SE Vaccine

ID93 + GLA-SE is a novel subunit TB vaccine candidate. The recombinant fusion protein ID93 comprises four antigens associated with virulence (Rv2608, Rv3619, and Rv3620) or latency (Rv1813). These antigens elicited dominant Th1 responses associated with reduced bacterial burden in animal models. Twelve ESX-1 family members including Rv3619 and Rv3620 are unique to *M. tuberculosis* with no homologues in *M. bovis* or BCG, whereas Rv1813 and Rv2608 are expressed by both *M. bovis* and BCG. ID93 is formulated in a synthetic toll-like receptor 4 agonist in a stable oil-in-water emulsion known as glucopyranosyl lipid, a stable emulsion (GLA-SE) formulation. Nicholson et al. described in their paper [22], that ID93 + GLA-SE is efficacious, providing prophylactic protection in challenges against laboratory-adapted, drug-resistant, or hypervirulent Beijing clinical *M. tuberculosis* isolates. ID93 + GLA-SE also showed utility as a therapeutic vaccine in mice and NHPs [22]. In the randomized double-blind placebo-controlled phase 1 trial, they enrolled HIV-negative previously BCG-vaccinated adults with no evidence of previous or current TB disease from community volunteers in the Worcester region of Western Cape, South Africa. ID93 + GLA-SE was well tolerated, and no serious vaccine-related adverse events were recorded. These data support efficacy testing of two administrations of the lowest ID93 vaccine dose (2 μg) in TB-endemic populations [22].

9.2 H56 Vaccine

In a mouse model in which H56 (Ag85B-ESAT-6-Rv2660) TB vaccine candidate was used, it was found that low vaccine antigen doses are required for optimal post-exposure vaccine protection, but not for preventive vaccine protection [7]. Further, it was reported that loss of protection from high-dose post-exposure vaccination is not associated with loss of overall vaccine response magnitude but is associated with high differentiation and low functional avidity of vaccine-specific CD4 T cells [7].

9.3 HVJ-E/HSP65 DNA + IL-12 DNA Vaccine

Developing a vaccine that can prevent reactivation in LTBI individuals would greatly impact the global TB burden.

Furthermore, effective therapeutic vaccination would be essential for treating MDR-TB and XDR-TB with low responsiveness to second-line antibiotics.

Therefore, in our previous study, we first established vaccine protection in post-exposure or therapeutic animal (mouse and cynomolgus monkey) models cited by Billeskov et al. [5, 7]. There have also been other studies on therapeutic vaccines. Aagaard et al. described a multistage TB vaccine that confers efficient protection before and after exposure [8]. They reported that the H56 subunit vaccine, a fusion protein incorporating Ag85B, ESAT-6, and Rv2660c, showed high levels of activity when administered after exposure in the Cornell model [8].

10 Conclusion (TB Vaccines Available in the Future)

First, several vaccines have already shown clinical efficacy. In other words, several vaccine candidates have already proven their safety and immunogenicity. Second, there have been several positive signals from clinical trials conducted in previous years; these include proof of concept that a subunit vaccine can partially protect against active TB when administered to individuals with LTBI after exposure [19, 25]. Third, BCG revaccination of *Mtb*-unexposed individuals has provided indirect evidence for partial prevention of sustained *Mtb* infection [20]. BCG revaccination outcome was determined using IGRA, which measures T cell immune responses rather than *Mtb* infection [29].

Promising results from candidates such as M72/AS01$_E$, VPM1002, and H56:IC31 point to a brighter future in the field of TB vaccine development [27].

Therapeutic vaccine candidates may shorten the period of treatment with chemotherapeutic drugs and may have remarkable efficacy in patients with TB who have drug allergies [8, 22, 23].

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References

1. WHO: Global tuberculosis report 2020.
2. Yoshida S, Tanaka T, Kita Y, Kuwayama S, Kanamaru N, Muraki Y, et al. DNA vaccine using hemagglutinating virus of Japan-liposome encapsulating combination encoding mycobacterial heat shock protein 65 and interleukin-12 confers protection against mycobacterium tuberculosis by T cell activation. Vaccine. 2006;24:1191–204.; PMID: 16216394. https://doi.org/10.1016/j.vaccine.2005.08.103.
3. Okada M, Kita Y, Nakajima T, Kanamaru N, Hashimoto S, Nagasawa T, et al. Evaluation of a novel vaccine (HVJ-liposome/HSP65DNA+IL-12 DNA) against tuberculosis using the cynomolgus monkey model of TB. Vaccine. 2007;25:2990–3.; PMID: 17280753. https://doi.org/10.1016/j.vaccine.2007.01.014.
4. Okada M, Kita Y. Tuberculosis vaccine development: the development of novel (preclinical) DNA vaccine. Hum Vaccin. 2010;6:297–308.; PMID: 20372079. https://doi.org/10.4161/hv.6.4.10172.
5. Okada M, Kita Y, Nakajima T, Kanamaru N, Hashimoto S, Nagasawa T, et al. Novel prophylactic and therapeutic vaccine against tuberculosis. Vaccine. 2009;27:3267–70.; PMID: 19200841. https://doi.org/10.1016/j.vaccine.2009.01.064.
6. Kaneda Y, Nakajima T, Nishikawa T, Yamamoto S, Ikegami H, Suzuki N, et al. Hemagglutinating virus of Japan (HVJ) envelope vector as a versatile gene delivery system. Mol Ther. 2002;6:219–26.; PMID: 12161188. https://doi.org/10.1006/mthe.2002.0647.
7. Billeskov R, Lindenstrøm T, Woodworth J, Vilaplana C, Cardona P-J, Cassidy JP, et al. High antigen dose is detrimental to post-exposure vaccine protection against tuberculosis. Front Immunol. 2018;8:1973.; PMID: 29379507. https://doi.org/10.3389/fimmu.2017.01973.
8. Aagaard C, Hoang T, Dietrich J, Cardona P-J, Izzo A, Dolganov G, et al. A multistage tuberculosis vaccine that confers efficient protection before and after exposure. Nat Med. 2011;17:189–94.; PMID: 21258338. https://doi.org/10.1038/nm.2285.
9. Okada M, Kita Y, Nakajima T, Kanamaru N, Hashimoto S, Nagasawa T, et al. Novel prophylactic vaccine using a prime-boost method and hemagglutinating virus of Japan-envelope against tuberculosis. Clin Dev Immunol 2011; 2011:549281; PMID: 21437226; https://doi.org/10.1155/2011/549281.
10. Kita Y, Tanaka T, Yoshida S, Ohara N, Kaneda Y, Kuwayama S, et al. Novel recombinant BCG and DNA-vaccination against tuberculosis in a cynomolgus monkey model. Vaccine. 2005;23:2132-2135; PMID:15755583 https://doi.org/10.1016/j.vaccine.2005.01.057.
11. Okada M, Kita Y, Nakajima T, Kanamaru N, Hashimoto S, Nagasawa T, et al. Novel therapeutic vaccine: granulysin and new DNA vaccine against tuberculosis. Hum Vaccin. 2011;7(Suppl):60–7.; PMID: 21546794. https://doi.org/10.4161/hv.7.0.14563.
12. Okada M, Kita Y, Nakajima T, Hashimoto S, Nakatani H, Nishimatsu S, et al. The study of novel DNA vaccines against tuberculosis: induction of pathogen-specific CTL in the mouse and monkey models of tuberculosis. Hum Vaccin Immunother. 2013;9:515–25.; PMID: 23249543. https://doi.org/10.4161/hv.23229.
13. Kita Y, Okada M, Nakajima T, Kanamaru N, Hashimoto S, Nagasawa T, et al. Development of therapeutic and prophylactic vaccine against tuberculosis using monkey and transgenic mice models. Hum Vaccin 2011; 7 Suppl:108–114; PMID: 21263229; https://doi.org/10.4161/hv.7.0.14571.
14. Lowrie DB. DNA vaccines against tuberculosis. Curr Opin Mol Ther. 1999;1:30–3. PMID: 11249680
15. Flynn JL, Chan J. Immunology of tuberculosis. Annu Rev Immunol. 2001;19:93–129.; PMID: 11244032. https://doi.org/10.1146/annurev.immunol.19.1.93.

16. Kaufmann SHE, Lange C, Rao M, Balaji KN, Lotze M, Schito M, et al. Progress in tuberculosis vaccine development and host-directed therapies--a state of the art review. Lancet Respir Med. 2014;2:301–20.; PMID: 24717627. https://doi.org/10.1016/S2213-2600(14)70033-5.
17. Walsh GP, Tan EV, dela Cruz EC, Abalos RM, Villahermosa LG, Young LJ, et al. The Philippine cynomolgus monkey (Macaca fasicularis) provides a new nonhuman primate model of tuberculosis that resembles human disease. Nat Med. 1996;2:430–6.; PMID: 8597953. https://doi.org/10.1038/nm0496-430.
18. Andersen P, Scriba TJ. Moving tuberculosis vaccines from theory to practice. Nat Rev Immunol. 2019;19:550–62.; PMID: 31114037. https://doi.org/10.1038/s41577-019-0174-z.
19. Tait DR, Hatherill M, Van Der Meeren O, Ginsberg AM, Van Brakel E, Salaun B, et al. Final analysis of a trial of M72/AS01$_E$ vaccine to prevent tuberculosis. N Engl J Med. 2019;381:2429–39.; PMID: 31661198. https://doi.org/10.1056/NEJMoa1909953.
20. Nemes E, Geldenhuys H, Rozot V, Rutkowski KT, Ratangee F, Bilek N, et al. Prevention of M. tuberculosis infection with H4:IC31 vaccine or BCG revaccination. N Engl J Med. 2018;379:138–49.; PMID: 29996082. https://doi.org/10.1056/NEJMoa1714021.
21. Suliman S, Luabeya AKK, Geldenhuys H, Tameris M, Hoff ST, Shi Z, et al. Dose optimization of H56:IC31 vaccine for tuberculosis-endemic populations. A double-blind, placebo-controlled, dose-selection trial. Am J Respir Crit Care Med. 2019;199:220–31.; PMID: 30092143. https://doi.org/10.1164/rccm.201802-0366OC.
22. Penn-Nicholson A, Tameris M, Smit E, Day TA, Musvosvi M, Jayashankar L, et al. Safety and immunogenicity of the novel tuberculosis vaccine ID93 + GLA-SE in BCG-vaccinated healthy adults in South Africa: a randomised, double-blind, placebo-controlled phase 1 trial. Lancet Respir Med. 2018;6:287–98.; PMID: 29595510. https://doi.org/10.1016/S2213-2600(18)30077-8.
23. Okada M, Kita Y, Hashimoto S, Nakatani H, Nishimastu S, Kioka Y, et al. Preclinical study and clinical trial of a novel therapeutic vaccine against multi-drug resistant tuberculosis. Hum. Vaccin Immunother. 2017;13:298–305.; PMID: 27960629. https://doi.org/10.1080/21645515.2017.1264781.
24. Reed SG, Alderson MR, Dalemans W, Lobet Y, Skeiky YAW. Prospects for a better vaccine against tuberculosis. Tuberculosis. 2003;83:213–9.; PMID: 12758214. https://doi.org/10.1016/s1472-9792(02)00080-x.
25. Van Der Meeren O, Hatherill M, Nduba V, Wilkinson RJ, Muyoyeta M, Van Brakel E, et al. Phase 2b controlled trial of M72/AS01$_E$ vaccine to prevent tuberculosis. N Engl J Med. 2018;379:1621–34. https://doi.org/10.1056/NEJMoa1803484.
26. Rodo MJ, Rozot V, Nemes E, Dintwe O, Hatherill M, Little F, et al. A comparison of antigen-specific T cell responses induced by six novel tuberculosis vaccine candidates. PLoS Pathog. 2019;15:e1007643.; PMID: 30830940. https://doi.org/10.1371/journal.ppat.1007643.
27. Tameris MD, Hatherill M, Landry BS, Scriba TJ, Snowden MA, Lockhart S, et al. Safety and efficacy of MVA85A, a new tuberculosis vaccine, in infants previously vaccinated with BCG: A randomised, placebo-controlled phase 2b trial. Lancet. 2013;381:1021–8.; PMID: 23391465. https://doi.org/10.1016/S0140-6736(13)60177-4.
28. Hansen SG, Zak DE, Xu G, Ford JC, Marshall EE, Malouli D, et al. Prevention of tuberculosis in rhesus macaques by a cytomegalovirus-based vaccine. Nat Med. 2018;24:130–43.; PMID: 29334373. https://doi.org/10.1038/nm.4473.
29. Kaufmann SHE. Vaccination against tuberculosis: revamping BCG by molecular genetics. Guided by immunology. Front Immunol. 2020;11:316.; PMID: 32174919. https://doi.org/10.3389/fimmu.2020.00316.
30. Darrah PA, Zeppa JJ, Maiello P, Hackney JA, Wadsworth MH 2nd, Hughes TK, et al. Prevention of tuberculosis in macaques after intravenous BCG immunization. Nature. 2020;577:95–102.; PMID: 31894150. https://doi.org/10.1038/s41586-019-1817-8.

Chapter 12
Clinical Trials of TB: Challenges and Opportunities

Lorenzo Guglielmetti and C. Robert Horsburgh Jr.

Abstract TB was the subject of one of the first randomized, controlled clinical trials in 1946, beginning a series of studies that led to a highly effective 6-month four-drug regimen for TB treatment by 1976. Since that time, the emergence of drug resistance and challenges in ensuring completion of the full 6 months of treatment have raised new issues that need to be addressed by clinical trials. Beginning in 2000, several new classes of antimycobacterial agents have become available, and a series of innovative clinical trials have focused on shortening the treatment of drug-susceptible diseases and improving cure rates for drug-resistant diseases. This chapter will review recent trial results and summarize ongoing studies. In addition, it will discuss challenges to current and future trials and new analytic approaches to address these challenges.

Keywords Tuberculosis · Clinical trial

L. Guglielmetti
Médecins Sans Frontières, Paris, France

Centre d'Immunologie et des Maladies Infectieuses, Sorbonne – Université, INSERM, (U1135 – E2), Paris, France

Centre National de Référence des Mycobactéries et de la Résistance des Mycobactéries aux antituberculeux, Bactériologie-Hygiène, AP-HP.Sorbonne Université, Site Pitié, Paris, France

C. Robert Horsburgh Jr. (✉)
Departments of Epidemiology, Biostatistics, Global Health and Medicine, Boston University Schools of Public Health and Medicine, Boston, MA, USA
e-mail: rhorsbu@bu.edu

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1 Introduction

1.1 History of Trials of TB Treatment

TB has long been a disease whose treatment was based on the highest level of evidence [1]. Indeed, the two studies promoted by the British Medical Research Council (MRC) in 1946 and 1947 on the use of streptomycin for meningeal and pulmonary TB are among the first randomized controlled clinical trials in the history of medicine. These studies showed that 4–6 months of daily streptomycin treatment, compared to bed rest, led to greater radiologic and microbiologic improvement and reduced mortality. However, the acquisition of resistance to streptomycin was prompt and almost universal, and the mortality reduction was lost after long-term follow-up. In 1948, the co-administration of 3 months of streptomycin and para-amino-salicylic acid was shown to prevent drug resistance to streptomycin, establishing the paradigm of combination treatment for TB. After 1952, these findings were confirmed in treatment regimens containing a new anti-TB drug, isoniazid. Subsequent trials tested triple-drug therapy and introduced the concept of treatment phases: an "intensive" phase with two oral drugs and one injectable, streptomycin, followed by a "continuation" phase with two oral drugs. These trials also showed that relapse rates decreased with the increase in total anti-TB treatment duration, which needed to last at least 12 months. In addition, it was demonstrated that a residual lung cavity after 1 year of treatment required prolonged treatment to prevent relapse. In parallel, studies in the late 1950s provided strong evidence that ambulatory-based antimycobacterial treatment of patients living at home was as effective as sanatorium-based antimycobacterial treatment. From 1970 to 1982, a series of clinical trials tested "short-course" regimens (4–8 months) compared to the contemporary standard of care of at least 12, and up to 24 months of treatment. Many of these studies included two new highly-effective drugs, pyrazinamide (discovered in 1954) and rifampicin (discovered in 1963), in different combinations with isoniazid and streptomycin. Overall, the addition of pyrazinamide (for the first 2 months) and rifampicin allowed reduction of treatment duration to 6 months by preventing post-treatment relapse. Finally, the standardized short course regimen was defined in 1976 by the replacement of streptomycin with an oral drug, ethambutol. Following these major achievements, developed through a rigorous stepwise development approach by the MRC, the standard of care for rifampicin-susceptible TB was established for the next 4 decades.

In contrast to the development of treatment for drug-susceptible TB, the therapy of drug-resistant TB was historically neglected. For instance, strains with monoresistance to isoniazid have been treated for decades with the same standardized first-line regimen and the impact of such resistance on treatment outcomes was not carefully examined. Recently, however, it has been shown that isoniazid-monoresistant strains treated with the standard regimen have poorer outcomes and increased risk of acquiring rifampicin resistance [2, 3]. Since 2017, the WHO has recommended a specific treatment regimen for isoniazid monoresistance, including fluoroquinolone; however, these recommendations are based exclusively on

retrospective, observational evidence. To date, no clinical trials have examined the treatment of isoniazid-monoresistant TB.

In contrast to isoniazid resistance, the major impact of resistance to rifampicin on TB treatment outcomes has been clear since the earliest reports. However, the possibility of successfully treating rifampicin-resistant and, in particular, multidrug-resistant tuberculosis (MDR-TB), defined by resistance to both isoniazid and rifampicin, was initially questioned due to the lack of therapeutic alternatives, the excessive complexity of regimens based on poorly-tolerated second-line drugs, and the paucity of resources in high-incidence settings. Even as the need to provide optimal treatment for MDR-TB became evident, performing randomized clinical trials for this condition was considered challenging for multiple reasons: (a) the prolonged treatment duration, which increases cost and time to perform such studies; (b) the individualized nature of conventional MDR-TB treatment, which would complicate the design of a trial; and (c) the misconception that promoting clinical research on MDR-TB would "divert" the scarce available resources from drug-susceptible TB. As a result, current WHO recommendations on the treatment of MDR-TB were until recently of very low quality and based almost exclusively on observational data [4, 5].

The lack of political will and funding for TB clinical trials is even more notable for patients whose MDR-TB has additional resistance to fluoroquinolones, a class which is often considered the most effective among second-line drugs. Fluoroquinolone-resistant MDR-TB, also defined as pre-extensively drug-resistant (pre-XDR) TB according to 2021 WHO definitions, is associated with abysmal treatment outcomes. To date, no randomized controlled clinical trial results are available for this condition.

1.2 Overview of Recent TB Clinical Trial Results

In the last decade, an increasing number of clinical trials have provided important results in the field of TB. Remarkably, a few randomized clinical trials have even been completed for rifampin-resistant TB, including the first Phase III randomized clinical trial for this form of TB.

1.2.1 Clinical Trials of Drug-Susceptible Pulmonary TB

In 2014, three major Phase III randomized, controlled clinical trials reported results of the non-inferiority comparison between 4-month fluoroquinolone-based experimental regimens and the 6-month standard of care for drug-susceptible TB (Table 12.1). REMoxTB tested two 4-month experimental regimens where moxifloxacin, given for 4 months, replaced either ethambutol or isoniazid [6]. In OFLOTUB, ethambutol was replaced by a 4-month treatment with gatifloxacin [7]. RIFAQUIN tested two experimental arms: a 4-month regimen in which isoniazid was replaced by daily moxifloxacin for 2 months, followed by a 2-month continuation phase with moxifloxacin and rifapentine administered twice weekly; or a

Table 12.1 Summary of results of recent trials aiming to reduce treatment duration for drug-susceptible TB to 4 months

Trial 4-month regimen (s)	Relapse rate in standard 6-month arm	Relapse rate in 4-month arm	Non-inferiority?
REMoxTB • HRZMfx. • MfxRZE.	2.4%	8.2% 11.7%	Not met Not met
OFLOTUB • HRZGfx.	7.3%	14.8%	Not met
RIFAQUIN • MfxRZE/ MfxRpt.	3.2%	14.1%	Not met
NIRT 00018/9 • MfxHRZE. • GfxHRZE.	5.0%	8.9% 14.5%	Not met Not met
TBRU • HRZE.	1.5%	6.6%	Not met
S31/A5349 • HRptZE. • HRptZMfx.	3.3%	10.0% 6.0%	Not met Met

H isoniazid, *R* rifampicin, *Z* pyrazinamide, *Mfx* moxifloxacin, *E* ethambutol, *Gfx* gatifloxacin; *Rpt* rifapentine

6-month regimen in which isoniazid was replaced by daily moxifloxacin for 2 months, followed by a 4-month continuation phase with moxifloxacin and rifapentine administered weekly [8]. Despite previous pre-clinical and Phase II clinical trials supporting the use of fluoroquinolones to reduce the duration of drug-susceptible TB treatment, all shortened experimental arms in these three studies failed to achieve non-inferiority compared to the standard of care, calling into question the value of culture conversion as a surrogate marker for relapse-free cure [9]. A systematic review and meta-analysis including individual patient data of 3405 participants from these three trials have shown that, in subjects with limited TB disease (low sputum smear grade and/or absence of pulmonary cavities), 4-month regimens achieved non-inferiority compared to the control. Interestingly, this study also highlighted that, for subjects with a difficult-to-treat phenotype, 6 months of treatment may be insufficient to achieve optimal relapse-free cure rates [10].

In 2021, the results of Study 31/A5349, a drug-susceptible TB Phase III randomized controlled clinical trial with the same objective, were published, demonstrating the first successful 4-month treatment for drug-susceptible TB [11]. Based on promising results of a few Phase II trials evaluating the efficacy and safety of rifapentine, Study 31/A5349 tested two 4-month experimental arms including daily high-dose rifapentine instead of rifampicin. In addition, in one arm ethambutol was replaced by moxifloxacin given throughout the 4 months of treatment. While the 4-month regimen including rifapentine, moxifloxacin, isoniazid, and pyrazinamide achieved non-inferiority to the standardized 6-month control in both microbiologically eligible and assessable populations, the experimental regimen without moxifloxacin did not. Overall safety results were comparable between the non-inferior

experimental arm and the control. These results represent a major breakthrough and the first successful attempt at shortening TB treatment to less than 6 months.

1.2.2 Clinical Trials of Drug-Resistant Pulmonary TB

The first shorter treatment for rifampicin-resistant TB was recently added to WHO recommendations for a subgroup of selected patients. This recommendation was supported by the results of the first Phase III randomized, controlled clinical trial of patients with MDR-TB (STREAM Stage 1), which established non-inferiority of a standardized 9- to 12-month regimen comprising seven drugs compared to the conventional, individualized 18–24 month regimen [12].

More importantly, after decades of stagnation in development of drugs to treat TB, three new drugs from two different classes (bedaquiline, a diarylquinoline; delamanid and pretomanid, an imidazooxazole and a closely related imidazooxazine) have been approved by stringent regulatory authorities since 2012. The first of these, bedaquiline, was approved based on results of two Phase IIb/III studies: in these randomized controlled trials, patients with pulmonary MDR-TB were randomly assigned to receive either bedaquiline or placebo in combination with optimized background regimens (OBR) [13, 14]. Compared to the placebo arm, patients in the bedaquiline arm showed significantly shorter median times and higher rates of culture conversion in both studies. Furthermore, the rate of cure according to WHO treatment outcomes were higher in the bedaquiline arm: however, favorable outcomes reported in the control arm were very low (32%). Similarly, delamanid conditional approval was supported by a Phase IIb trial comparing 8-week culture conversion rates between delamanid plus OBR and placebo plus OBR, and a follow-up study, where participants were offered the opportunity to receive additional 6 months of open-label treatment with delamanid [15, 16]. While the experimental arms in these trials achieved higher culture conversion rates and more favorable long-term outcomes than control arms, the findings were not confirmed by a subsequent Phase III trial [17]. In that trial, 24 weeks of delamanid did not lead to a significant reduction in time to culture conversion (the primary outcome) compared to placebo, and outcomes were similar in the two groups; this was likely attributable to the fact that, in that study, the OBR was highly effective (77.2% successful outcomes at 30 months). The third new drug, pretomanid, was approved based on evidence provided by the NiX-TB study, a Phase III uncontrolled trial that tested a six-month combination of pretomanid, high-dose linezolid, and bedaquiline for pre-XDR and XDR-TB [18]. This regimen achieved excellent rates of relapse-free cure, although at the cost of substantial toxicity, mostly associated with linezolid. The small sample size and absence of a control arm, however, reduced the quality of evidence produced by the trial and lessened its impact on WHO recommendations, which only support the use of pretomanid as part of the NiX-TB combination under operational research conditions [4].

Finally, results from a Phase II trial, ACTG5343 (DELIBERATE), which tested the cardiac safety of bedaquiline and delamanid, have been recently published [19].

This trial focused on regular measurement of the QTc interval across three experimental arms: bedaquiline alone, delamanid alone, and their combination, in addition to an OBR. Overall, the bedaquiline-delamanid combination arm was associated, as expected, to a larger QTc increase during treatment: reassuringly, however, this did not translate in a significant increase of Grade 3/4 adverse events nor of deaths. This result should reduce concerns about the use of these two agents together [20].

The impact of the results of STREAM Stage 1 have been somewhat diminished by the concurrent evolution of the standard of care for the treatment of rifampicin-resistant TB, with the promotion of new/repurposed drugs and the demotion of second-line injectables. This has resulted in many TB programs substituting bedaquiline for the injectable in the STREAM regimen. The resulting regimens need better data on safety and efficacy, and both clinical trials and observational research studies are underway to evaluate them.

2 Challenges to Conducting Clinical Trials for Pulmonary TB

2.1 Design Challenges

The goal of a Phase III TB clinical trial is to determine if a treatment or treatment regimen is an improvement on currently existing treatments. This is most easily accomplished when there is no existing treatment, in which case the experimental treatment being evaluated is compared to no treatment (usually as a placebo). The object is to determine if the experimental treatment is *superior* to a control arm, in this case a placebo treatment. Where there is an existing treatment that is not very good (such as was the case in MDR-TB until recently), the control arm might be the standard therapy. The ability to determine superiority is related to the degree of superiority that is being sought and depends on the size of the trial. With an extremely large trial, a superiority of 1% better than the control could be demonstrated statistically, but this small degree of improvement would likely not be worth the very large cost. Thus, an improvement of 10–20% is usually sought.

When the existing treatment is very good, as in DS-TB treatment where 90–95% cures are obtained, it becomes impractical to seek to demonstrate superiority, since the experimental treatment cannot exceed 100%. In such cases, a new treatment might be preferred because it is shorter or better tolerated, and not worse than the existing treatment. Thus, it might be desirable to demonstrate efficacy *equivalent* to the existing treatment. However, statistical equivalence requires an immense sample size and is therefore not feasible. Instead, the trial is designed to demonstrate *non-inferiority* [21, 22]. This type of design is a practical approach that requires some assumptions which introduce inherent but unavoidable weaknesses.

The first challenge is the selection of a “non-inferiority (NI) margin.” This is the acceptable difference between the efficacy of the experimental regimen and that of

the control regimen. There is no hard and fast rule for this, and it is determined by consensus. Currently, the NI margin for DS-TB trials is accepted to be ~6% and that for DR-TB trials to be ~12%, reflecting the fact that DR-TB regimens are usually less effective than DS-TB regimens. The weakness of this approach is that the experimental regimen might actually be slightly less effective than the control regimen, and in successive trials one might define it as non-inferior regimens that were actually inferior to the original control. To date, however, this has not been shown to be the case.

The second challenge is that non-inferiority only uses efficacy as its output. From the ethical point of view, a slightly less effective regimen that was better tolerated might result in more cures than one which was more effective but poorly tolerated. The standard "Experimental" clinical trial is focused only on the regimen and fails to take into account factors such as the willingness of patients to complete the regimen outside of a trial setting, as well as cost in money and time to the patient. Moreover, experimental trials are often affected by selection bias, in that patients who might have more difficulty adhering to a regimen are excluded. The result of this is that the results cannot be generalized to such patients, who comprise a substantial proportion of the affected population.

To address this issue, a different type of trial has been recommended, called a "Pragmatic" trial [23]. In such a trial, the experimental regimen is compared to a control in a programmatic situation, and no patients are excluded. Such trials are meant to occur after an Experimental trial has demonstrated that the regimen is efficacious and the goal is to demonstrate effectiveness. However, such trials are rarely conducted, since the regulatory imperative that drives demand for Experimental trials is no longer present.

Given the cost of clinical TB trials, a number of designs have sought to increase efficiency. This can be done by simultaneously comparing multiple experimental arms to a single control (without comparing the experimental arms to each other). Arms that do not meet predefined efficacy or tolerability criteria are dropped (in multi-arm multi-stage "MAMS" designs) or downsized (by adaptive randomization), so that resources are not expended on regimens that are less promising [24].

2.2 Statistical Challenges

Clinical trials are randomized to control for unmeasured confounders. However, when a trial is performed over multiple sites, this can become challenging. Blinding of randomization is essential to preclude inadvertent assignment of one subpopulation of participants to a particular arm. Randomization must be blocked by site to prevent differential allocation by site, which can preclude controlling for site, an important safeguard. When subgroups of interest (e.g., HIV-infected participants) vary by site, this may be difficult.

Sample size calculations are performed based on expected prevalence of risk factors and outcomes. This helps provide confidence that the study is likely to have

adequate power to confirm or refute the hypothesis being tested. However, sample size calculations are only estimates. If the actual enrollment into the study is different from what was expected, resizing a study may be required. In practice, this is usually suggested to the study investigators by the Independent Data Monitoring Committee (IDMC) in order to ensure that participants are not being enrolled in a study that will not yield useful information.

Most clinical studies specify two distinct analysis populations: "intention to treat (ITT)" and "per protocol (PP)." The ITT population includes all persons randomized. This maintains the ability of the study to control for unmeasured confounders, but sacrifices the ability to directly measure the effect of the regimen *if it had been taken,* since participants are included without regard to having received the doses. On the other hand, the PP population only includes those who completed the regimen (or some prespecified proportion of it) and gives a better assessment of the efficacy of the regimen, but at the expense of being less able to control for unmeasured confounders. For superiority trials, the ITT population is almost always the population analyzed for the primary outcome. For a non-inferiority trial, however, the ITT population may decrease the difference in effect seen between the two arms, falsely leading to a conclusion of non-inferiority. The consensus in the field is now that both populations should be examined before declaring non-inferiority.

In all clinical trials, an essential requirement is minimizing loss-to-follow-up (LTFU). Persons who fail to complete follow-up may represent missed study endpoints and are thus an important form of potential selection bias. Since adverse outcomes are often a small proportion of the overall study population, even 10% LTFU may jeopardize the ability of the study to draw robust conclusions [25, 26]. Thus, most TB trials focus substantial resources on ensuring that participants are able to complete the full follow-up and that all potential relapses during that period are carefully documented.

2.3 Challenges, Example 1 (Control Populations)

The inclusion of a control arm represents one of the main methodological strengths of a clinical trial: first, randomized allocation permits accounting for unmeasured confounders; second, the control arm provides an "internal," optimal comparator since it shares the main characteristics (study population, inclusion/exclusion criteria, geographical setting, time of implementation) with the experimental arm/s, thus ensuring that any difference in trial outcome/s can be attributed to treatment and not to conditions specific to the trial population. The control arm is usually the standard of care treatment (if it exists), thus allowing interpretation of study results in light of current practice and treatment policies. From an ethical standpoint, the control arm should ensure the best possible standard of care treatment [27].

The preferred approach is including an internal control population treated with the standard-of-care treatment regimen: the latter can be standardized or designed to follow a specific guideline document ("prescriptive" approach) or adapted according to changing international/national recommendations ("evolving" approach).

When an internal control is not possible, different approaches have been proposed with regard to the choice of the comparator population. Using an external comparator such as results from historical observational cohorts (as in the NiX-TB trial), is inherently biased [28]. A second approach is to compare a single drug versus placebo in addition to an optimized background regimen. This approach has the inconvenience of requiring a large sample size to show superiority of the experimental arm, in particular, if the comparator achieves high rates of favorable outcomes.

For trials that have included an internal control, specific challenges may arise when the standard of care changes during the trial's implementation, as exemplified by recent trials for drug-resistant TB. The state-of-the-art treatment of MDR- and XDR-TB has been revolutionized in recent years, and this has been reflected by the evolution of international recommendations: since 2011, the WHO has released more than 10 guidance documents on the treatment of drug-resistant TB.

In any case, investigators and the trial IDMC should carefully assess changes in the standard of care and evaluate the need for amendments to the study protocol and/or for informing study participants.

2.4 Challenges, Example 2 (Follow-Up Duration)

As outlined in the Introduction, the development of the current "short course" treatment for rifampicin-susceptible TB was pursued through a series of clinical trials that led to progressive shortening of the treatment duration from 18–24 to 6 months. These carefully conducted sequential trials included long post-treatment follow-up periods of 2–3 years to assess the relapse rate, which was often included in the primary trial outcome. Further efforts to shorten the treatment of both rifampicin-susceptible and rifampicin-resistant TB have confirmed the importance of relapses, which contributed to the failure of many fluoroquinolone-based DS-TB regimens to show non-inferiority [29].

However, the need for long post-randomization follow-up periods has a major impact on TB trials by increasing costs and complicating feasibility, and ultimately becoming an additional factor increasing the time to yield results. Indeed, 5–8 years usually elapse between enrollment start in large Phase III TB clinical trials and dissemination of results. In addition, participant retention for prolonged post-treatment periods is challenging. For these reasons, and in the absence of reliable biomarkers to predict the risk of relapse, the clinical research community has had substantial motivation to reduce the follow-up period.

It has been recently shown, by pooling data on 574 relapses (including both recurrence and reinfections) reported in 15 clinical trials, that the vast majority (78%) of relapses occurred during the first 6 months, and almost all (91%) during the first 12 months following treatment completion [30]. The authors postulated that these results supported stopping the trial 6 months after treatment completion of the last included participant while following up participants included previously up to 24 months post-randomization. A similar version of this "hybrid" follow-up

approach has been increasingly adopted by Phase III TB clinical trials, including in the field of drug-resistant TB (i.e., endTB and TB-PRACTECAL).

2.5 Challenges, Example 3 (Surrogate Markers)

The classic sequence of clinical research on new therapeutic compounds includes studies from Phase I–III (and IV). Studies in the earliest phases recruit smaller number of individuals, usually healthy subjects, to test safety of the compound, while later phases aim to assess efficacy in larger cohorts of patients. The specificity of the development pathway of new anti-TB drugs depends heavily on Phase II trials, which include Phase IIA studies testing early bactericidal activity of drugs or regimens given for a few days/weeks, and Phase IIB studies testing culture conversion rates (or time to culture conversion) during the first months of treatment. Subsequently, larger Phase III trials assess TB treatment outcomes and post-treatment relapse rates.

This traditional development pathway, however, has been shown to have important shortcomings [31]. First, following these steps for each drug in a new regimen requires a long time, which has been estimated at approximately 6 years per each drug if studied alone; developing a whole new regimen with this paradigm would therefore require decades.

Second, Phase II TB trials rely heavily on so-called "surrogate" markers for end-of-treatment outcomes. Surrogate markers are early indicators that correlate with the final clinical outcome (in the case of TB, relapse-free cure) and should be reproducible and have high predictive reliability. Unfortunately, surrogate markers identified and used to date in TB clinical development are suboptimal in serving the needs of trial Investigators and policymakers. A recent meta-analysis of Phase II TB trials has shown, for instance, that early bactericidal activity studies testing treatment for 1 week or less were not useful to compare different drugs and regimens [32]. Furthermore, early bactericidal studies evaluating 14 days of treatment were shown to be only capable of detecting very large differences between regimens due to wide confidence intervals linked to the usually small sample size of these studies. Recently, some studies have assessed early bactericidal activity over longer periods, up to 8 weeks. Phase IIB trials evaluating culture conversion are similarly inadequate to detect small differences between treatment regimens, in particular, in cases when only one drug in the regimen differs between experimental and control regimens, and when the comparator has a high rate of culture conversion, like for the current standard of care for DS-TB.

In addition, culture conversion has proven to be a poor predictor of treatment outcome and, in particular, of relapse-free cure. This has been clearly demonstrated, in DS-TB clinical research, by the discrepancy between Phase II studies, which reported the superiority in culture conversion rates of fluoroquinolone-containing regimens compared to the standard of care, and larger Phase III trials, where these regimens given for shorter duration failed to achieve non-inferiority because of

higher relapse rates. The lack of discrimination of culture conversion is particularly unfortunate, as this marker usually guides the choice of the best-performing experimental arm to be brought forward in multi-arm, multi-stage trials, and may therefore lead to suboptimal choices.

This issue has been particularly debated in the last decade, when two new drugs (bedaquiline and delamanid) were granted expedited approval for the treatment of MDR-TB by stringent regulatory authorities on the basis of Phase IIA/B trials assessing surrogate markers. Although justified by the dire need for new drugs for these difficult-to-treat forms of TB, these decisions have set a difficult precedent to follow [33]. Stringent regulatory authorities have tried to reduce the risks by providing a "conditional" recommendation, based on the engagement by manufacturers to perform Phase III trials assessing end-of-treatment outcomes and relapses: however, this recommendation is not binding and therefore may be ineffective.

2.6 Challenges, Example 4 (Pragmatic Trials)

Randomized controlled clinical trials are universally recognized as the gold standard to measure the efficacy and safety of medical interventions. However, the measured efficacy of the intervention does not always translate into a similar effectiveness once applied in routine care. This difference may be explained, at least partially, by the nature of clinical trials and the degree of control that is imposed on trial participants and investigators compared to what happens in clinical practice.

Usually, trials that aim at demonstrating a causal relationship between intervention and outcome, and at measuring the clinical and physiological effect of an intervention, are defined as "explanatory" or "experimental." In contrast, trials that assess the impact of the intervention in the context of routine care are defined as "pragmatic." While the former are the most suited to establish whether an intervention "works," the latter are the most useful for policymaking. These definitions may represent an over-simplification since, rather than a clear dichotomy, there is a continuum between these two approaches. The difference between explanatory and pragmatic designs may affect many aspects of the trials, including inclusion and exclusion criteria, sample size calculation, endpoints and outcomes, and analysis of the results.

Such differences are particularly relevant in the field of TB clinical research. In terms of trial population, explanatory trials tend to be stricter, including only patients with a microbiologically confirmed TB diagnosis and often only those with a positive sputum smear and/or culture at baseline. Similarly, patients with comorbidities, such as active HCV infection or HIV infection (especially with low CD4 lymphocyte counts), and special populations, such as pregnant or breastfeeding women or children, are often excluded. In contrast, pragmatic trials tend to include the vast majority of patients, including those that are treated empirically, as they would receive treatment in routine care. Another important difference is the choice of the comparator, which is usually standardized (including often a placebo) in explanatory

trials, but more flexible and allowed to evolve in pragmatic trials (see Example 1). Finally, outcome measurements may also vary according to the trial's approach, as pragmatic trials typically adopt outcome definitions that are simpler, based on clinical assessments, and more similar to program-defined patient outcomes.

When pragmatic trials capable of informing policymaking are lacking, their absence is often addressed by observational research, as exemplified by the evolution of WHO recommendations for the treatment of DR-TB and in particular for the use of new drugs, bedaquiline and delamanid. Initially, WHO guidelines adopted a conservative approach, informed by Phase II pivotal explanatory trials, where these drugs were recommended only for specific populations, for a standardized duration, and with restricted indications. With the increasing number of observational studies available, WHO has revised these recommendations and allows for broader, less strict access to the drugs. As a consequence, however, the evidence base for these guidelines is of very low quality and the recommendations are conditional.

2.7 Challenges, Example 5 (Non-inferiority Design)

When a non-inferiority design is used in a trial of Latent Tuberculosis Infection (LTBI) treatment, some unique challenges arise. Here the observed outcome is not a success, but failure, as indicated by the development of TB disease. As regimens become more effective, the observable endpoints decrease, leading to larger and larger sample sizes. This places increased pressure on the need to minimize LTFU. In addition, a non-inferiority trial of LTBI treatment faces a unique type of selection bias: rather than failure to include some persons who should have been included, the risk is including persons not at risk for the outcome. This recently occurred in a trial of a one-month regimen for LTBI [34]. In this trial, not all persons enrolled had LTBI; thus, they were not at risk for the outcome of TB disease (except if they were exposed after the regimen has been taken, which could not have been prevented by the treatment). Inclusion of such persons in the analysis population biases toward a null result, since they were not at risk and therefore could not experience the outcome. This could falsely lead to a conclusion of non-inferiority [35]. It is not clear if this actually led to an erroneous conclusion, since the difference between the two arms when the analysis was limited to persons who had been shown to have LTBI was small; however, the sample of such participants was too small to support a statistical conclusion of non-inferiority.

2.8 Challenges, Example 6 (Transition from Phase 2 to Phase 3)

A critical unresolved challenge is how to identify the best regimens to move forward into a phase 3 trial. Animal model data, largely from murine studies, do not provide adequate information because lack of *M. tuberculosis* persistence in the mouse precludes insight into relapse; moreover, human drug pharmacokinetics are

substantially different from those in the mouse. The traditional method has been to perform a smaller human Phase II trial with a surrogate endpoint, most commonly 2-month culture conversion, and then apply the proportion of improvement to the Phase III outcome of relapse-free cure. Unfortunately, the correlation between two-month culture conversion and relapse-free cure is poor [36] and this strategy has led to a number of failed Phase III trials [6–8, 37].

More recently, a new type of Phase II study design has been proposed, the "Phase IIC Selection Trial with Extended Post-treatment follow-up (STEP)." In this hybrid Phase II/III trial design, TB patients are given the experimental regimen for the duration for which it will be studied in Phase III and patients are followed for treatment failure and relapse for a fixed time period, usually 12 months [38]. This is essentially a pilot study that can provide data from which to estimate the likely relapse-free cure proportion and the sample size necessary to establish its efficacy. Such a pilot study can be designed to transition directly into a Phase III study, allowing the pilot study participants to contribute to the confirmatory Phase III study population. However, such adaptive design is limited by the long duration of follow-up currently required to observe the primary outcome. The result is a long delay between completion of the pilot and initiation of the full study.

3 Current TB Clinical Trials

3.1 Overview

Overall, the global pipeline of clinical research on the treatment of active pulmonary TB is evolving rapidly. The vast majority of these trials aim to show non-inferiority of a standardized experimental regimen with a shorter treatment duration compared to a control, which is standardized for drug-susceptible TB and often individualized for rifampicin-resistant TB.

Table 12.2 shows current/planned trials for drug-susceptible TB. These efforts mainly focus on four different approaches to achieve treatment shortening: (1) adding a fluoroquinolone to the standard regimen (Beijing Chest Hospital trial), similarly to previous trials that failed to show-non inferiority [29]; (2) increasing the dose of rifampicin, up to 30 mg/kg (RIFASHORT); (3) replacing rifampicin with high-dose rifapentine, similarly to the successful S31/A5349 trial, and adding clofazimine with a loading dose (CLO-Fast); (4) using new/repurposed drugs (bedaquiline, clofazimine, linezolid, pretomanid) in combination with fluoroquinolones and first-line drugs (SimpliciTB, TRUNCATE-TB). The duration of the shorter, experimental regimens in these trials ranges from 2 to 4 months. The trial design is conventional in most cases, with participants assigned to two to three treatment arms through balanced randomization; an exception is represented by TRUNCATE-TB, a MAMS trial. Finally, the PredictTB trial is testing the hypothesis that PET/CT scan at baseline and after 1 month of treatment can help identify participants who could be cured with a shorter treatment duration.

Table 12.2 Major ongoing and planned Phase II/III clinical trials for adult patients with active pulmonary drug-susceptible TB

Trial	Phase	Control arm	Country	R	Experimental treatment					Experimental treatment duration (months)	Clinicaltrials.gov Identifier (reference)
					Rpt	E	H	Z	Others		
Beijing Chest Hospital	4	Yes	China	X		X	X	X	Lfx	4,5	NCT02901288 [42]
CLO-Fast (ACTG 5362)	2	Yes	Multi		X	X	X	X	Cfz	3	NCT04311502
PredictTB[a]	2	Yes	Multi	X		X	X	X		4	NCT02821832
RIFASHORT	3	Yes	Multi	X		X	X	X		4	NCT02581527
SimpliciTB[b]	2/3	Yes	Multi					X	Bdq, Mfx, Ptm	4	NCT03338621
TRUNCATE-TB	3	Yes	2–3	X	X	X	X	X	Bdq, Cfz, Lzd	2–3[c]	NCT03474198

R rifampicin, *Rpt* rifapentine, *E* ethambutol, *H* isoniazid, *Z* pyrazinamide, *Lfx* levofloxacin, *Cfz* clofazimine, *Bdq* bedaquiline, *Mfx* moxifloxacin, *Ptm* pretomanid, *Lzd* linezolid

[a]Recruitment in the experimental arm has been interrupted prematurely following a DSMB review

[b]Includes a non-randomized arm recruiting patients with rifampicin-resistant TB

[c]Treatment duration is dependent on symptoms and sputum smear status at 8 weeks

Table 12.3 shows current/planned trials for rifampicin-resistant TB. While the majority of these trials include only fluoroquinolone-susceptible (i.e., DRAMATIC) or fluoroquinolone- and injectable-susceptible patients (i.e., STREAM 2), others (like TB-PRACTECAL) include all rifampicin-resistant patients regardless of fluoroquinolone susceptibility. Another trial, BEAT Tuberculosis, includes all rifampicin-resistant patients and adapts the treatment regimen to the result of rapid molecular tests for fluoroquinolone resistance. Experimental treatment regimens include in most cases the combination of at least one new drug (bedaquiline and/or delamanid or pretomanid), one or two repurposed drugs (clofazimine and linezolid), and a fluoroquinolone (levofloxacin or moxifloxacin), for a duration of 4 to 12 months. Pyrazinamide is also part of the experimental regimen in a substantial number of trials. Overall, "novel" trial designs are more frequent among trials for rifampicin-resistant than for those for drug-susceptible TB, including adaptive design (i.e., endTB), multi-arm multi-stage (i.e., TB-PRACTECAL), duration-randomized (i.e. DRAMATIC) designs, and trials testing a treatment strategy rather than a regimen (i.e., BEAT Tuberculosis). Additional details on some of these trial designs are provided below. Two trials (GRACE-TB and InDEX) compare individualized treatment guided by next generation sequencing to the standard of care.

Table 12.4 shows current/planned trials for rifampicin- and fluoroquinolone-resistant TB. Clinical trials specifically targeting this population, whose treatment is particularly challenging, are unfortunately rare. Moreover, out of three trials, only one includes an internal control arm. The experimental regimens include

Table 12.3 Major ongoing and planned Phase II/III clinical trials for adult patients with active pulmonary rifampicin-resistant TB

Trial	Phase	Control arm	Country	FQ	Experimental treatment regimen						Experimental treatment duration (months)	Clinicaltrials.gov Identifier (reference)
					Bdq	Dlm	Ptm	Lzd	Cfz	Other		
BEAT-TB	3	Yes[a]	South Africa	X	X	X		X	X		6	NCT04062201
DRAMATIC[b]	2	No	Multi	X	X	X		X	X		4–9[c]	NCT03828201
endTB[b]	3	Yes	Multi	X	X	X		X	X	Z	9	NCT02754765 [43]
GRACE-TB	NS	Yes[a]	China	X				X	X	Am, Cs, E, Pto, Z	NS	NCT03604848
InDEX	4	Yes	South Africa	X	X	X	X	X	X		NS	NCT03237182
MDR-END[b]	2	Yes	South Korea	X		X		X		Z	9–12[d]	NCT02619994 [44]
NeXT[e,f]	3	Yes[a]	South Africa	X	X			X		Eto, H, Tzd, Z	6–9[d]	NCT02454205
STREAM 2[f]	3	Yes	Multi	X	X				X	E, H, Pto, Z	9	NCT02409290 [45]
TB-PRACTECAL	3	Yes	Multi	X	X		X	X	X		6	NCT02589782
TB-TRUST[f]	3	Yes[a]	China	X				X	X	Cs, Z	6–9[g]	NCT03867136

FQ any fluoroquinolone, *Bdq* bedaquiline, *Dlm* delamanid, *Ptm* pretomanid, *Lzd* linezolid, *Cfz* clofazimine, *NS* not specified, *Z* pyrazinamide, *Am* amikacin, *Eto* ethionamide, *H* isoniazid, *Tzd* terizidone, *E* ethambutol, *Pto* prothionamide, *Cs* cycloserine

[a]Standardized control arm

[b]Includes participants with fluoroquinolone-susceptible TB

[c]Treatment duration is assigned randomly

[d]Treatment duration is dependent on sputum smear/culture conversion

[e]Recruitment has been interrupted prematurely

[f]Includes participants with fluoroquinolone-, second-line injectable-susceptible TB

[g]Treatment duration is prolonged in case of pyrazinamide resistance

Table 12.4 Major ongoing and planned Phase II/III clinical trials for adult patients with active pulmonary rifampicin-, fluoroquinolone-resistant TB

Trial	Phase	Control arm	Country	Experimental treatment					Experimental treatment duration (months)	Clinicaltrials.gov identifier (reference)
				Bdq	Dlm	Ptm	Lzd	Cfz		
BEAT-Tuberculosis	3	No	India	X	X		X	X	6–9[a]	NA
endTB-Q	3	Yes	Multi	X	X		X	X	6–9[b]	NCT03896685
ZeNIX	3	No	Multi	X		X	X		6	NCT03086486

Bdq bedaquiline, *Dlm* delamanid, *Ptm* pretomanid, *Lzd* linezolid, *Cfz* clofazimine, *NA* not available
[a]Treatment duration is assigned according to sputum culture conversion after 2 months of treatment
[b]Treatment duration is assigned according to baseline patient characteristics and sputum culture results during treatment

bedaquiline, a nitroimidazole/nitroimidazooxazine (delamanid or pretomanid), and linezolid, with or without clofazimine, administered for a duration that varies between 6 and 9 months.

Overall, advances in methodology are highly needed to help expedite late-stage regimen trials and optimize resources allocated to clinical research [39]. Below, we provide examples of ongoing or planned clinical trials that apply ground-breaking designs to improve the efficiency of the study and the impact of expected results.

3.2 Example 1 (Adaptive Trial Design: endTB)

In recent years, Bayesian adaptive clinical trials have been increasingly used in the fields of oncology and cardiology. The principle underlying these designs is the use of data generated by the trial itself to periodically modify randomization odds to favor experimental arms which perform better.

An ongoing Phase III clinical trial of MDR-TB, endTB, represents the first example of adaptive trial design in TB clinical research. In this trial, randomization is outcome adaptive using interim Bayesian analyses of efficacy endpoints [40, 41]. The initial randomization list used fixed block sizes with balanced allocation to all six arms (five experimental, one control). After approximately 30 participants are assigned to each arm, the response adaptation begins. At monthly intervals, interim treatment efficacy (at 8 and 39 weeks) is estimated for each arm, relative to the control, through Bayesian modelling. Randomization probabilities are updated after each interim analysis; a higher probability of randomization is assigned to arms with a greater interim treatment effect. The probability of assignment to the control arm matches the probability of assignment to the most effective experimental arm. The interim analysis is conducted by a Bayesian statistician who is not involved in study operations.

The implementation of this design has multiple advantages. First, it allows reduction of the total sample size. The endTB trial aims to recruit 750 participants

and is powered to show non-inferiority of up to three experimental arms compared to the control: to achieve a similar result with a traditional design with balanced randomization, a sample size of approximately 1100 participants would be required. Therefore, the endTB trial has the potential to identify multiple effective experimental regimens, rather than only one: this could increase the impact of the trial results, considering that patient characteristics, additional drug resistance of *M. tuberculosis* strains, and additive toxicities/interactions of concomitant medicines often require multiple alternatives for the treatment of rifampicin-resistant TB. Second, the adaptive design allows prospective participants to benefit from the most effective treatment regimen, according to early efficacy endpoints. These advantages, however, rely on the assumption of a correlation between early and late treatment outcomes (i.e., efficacy at early endpoints prevents relapses). In addition, the accrual of participants should not be too rapid, in order to allow for the adaptation to have a meaningful impact on numbers of randomized patients in each experimental arm.

3.3 Example 2 (Duration Randomized Design: DRAMATIC)

A recently initiated Phase II TB clinical trial seeks to address the challenge in transitioning from Phase II to Phase III by studying different durations of the same regimen and modeling the outcomes to identify the duration required to achieve a particular relapse-free cure proportion. Two hundred twenty participants with fluoroquinolone-susceptible MDR-TB will be randomized 1:1:1:1 to treatment for 16, 24, 32, or 40 weeks with a 5-drug regimen of bedaquiline, delamanid, linezolid, clofazimine, and levofloxacin. The results will be used to derive the relationship between duration and cure proportion. Thus, a duration that was not directly studied could be the one which yields 85%, 90%, or 95% relapse-free cure.

Compared to studying a single duration of a new treatment regimen, duration randomization has the advantage of avoiding the result that the duration selected was either too short (and thus not effective enough) or too long (and thus incurring excess toxicity). By following patients to observe relapse, this design provides important information for selection of the optimal treatment duration for study in a Phase III trial. The Phase II investigational durations are chosen to range from the shortest duration that could be expected to be successful to the longest one that is expected to achieve the maximal proportion of successful outcomes. In the traditional algorithm for arriving at the optimal duration, each of these durations would be studied independently to determine the shortest duration of the investigational regimen that was non-inferior to a standard treatment, an approach that has been recognized to be time consuming and inefficient, requiring a series of sequential studies. The duration-randomized design is also considerably more efficient than four parallel Phase 2c studies, as each arm contributes results to the model.

This design also lends itself to risk stratification, in that specific subgroups, such as participants with cavitary disease or HIV coinfection, may have a different relationship between duration and cure proportion. The model may demonstrate that such patients need a longer duration of treatment to reach the same cure proportion and give an indication of how much longer that treatment should be. This could greatly increase both the efficiency and the precision of future Phase III trials.

3.4 Example 3 (Strategy Trials: BEAT Tuberculosis and endTB-Q)

A different approach to clinical trial design is represented by testing a strategy, rather than a treatment regimen. Two recent clinical trials of rifampicin-resistant TB offer examples of this approach.

BEAT Tuberculosis is a Phase III trial randomizing rifampicin-resistant TB patients 1:1 to a combination of a fluoroquinolone (levofloxacin) with other four new/repurposed drugs (bedaquiline, clofazimine, delamanid, linezolid) or to a control arm containing the local standard-of-care treatment. The pragmatic principle of the trial is to start the regimen for all participants with rifampicin resistance in the experimental arm, and to adapt it when the results of rapid molecular testing for fluoroquinolones will be available. When such results are available, clofazimine will be dropped in participants with fluoroquinolone-susceptible TB, while levofloxacin will be dropped in participants with fluoroquinolone-resistant TB. This approach considers the delays that are often encountered in clinical practice, in particular, in resource-limited settings, and allows patients to benefit from rapid treatment initiation with a robust five-drug combination.

endTB-Q is a Phase III trial randomizing rifampicin-, fluoroquinolone-resistant TB patients 2:1 to a four-drug combination of bedaquiline, clofazimine, delamanid, and linezolid, or to a control arm with an individualized regimen designed according to latest WHO recommendations. The duration of treatment for participants randomized to the experimental arm can be 24 or 39 weeks and is determined according to the following: (1) extent of TB disease at baseline, defined by the presence of any cavitation on chest X-ray and by the quantitative measure of sputum smear microscopy results, and (2) treatment response, defined by sputum culture results at week 8 of treatment and later. In practice, patients with a limited extent of TB disease at baseline (smear-negative or smear-positive 1+ with no cavitation on chest X-ray) and sputum culture conversion obtained at week 8 of treatment (with no positive sputum culture results in the following weeks) will be treated for 24 weeks, while the others will receive 39 weeks of treatment. Such a precision medicine approach is supported by a recent meta-analysis of Phase III clinical trials for drug-susceptible TB, which has shown that baseline characteristics may be useful to identify subgroups of patients for whom treatment shortening is possible [10].

4 Future Perspectives

The current TB clinical research pipeline, including landmark trials summarized in Tables 12.2, 12.3, and 12.4, contains more than 30 ongoing/planned clinical trials. Despite TB research still being largely underfunded compared to the estimated need, it is reassuring to see that many studies are being conducted to improve the standard of care for TB treatment. It is noteworthy that many of these trials are being conducted in previously neglected areas, including trials for rifampicin-resistant TB and the first randomized controlled trials for isoniazid-monoresistant TB (FIRST, ACTG5373) and fluoroquinolone-resistant MDR-TB (endTB-Q). However, compared to the history of development of the short course treatment for drug-susceptible TB, these trials lack the systematic and stepwise development process that characterized MRC studies. Processes and evaluations are often significantly different between different trial sponsors, and treatment outcomes are increasingly complex and lack standardization. Efforts are being conducted to harmonize trial conduct, implementation, and assessments, and will hopefully lead to increased standardization in forthcoming years [46].

Innovations in design have become increasingly common in TB trials, allowing optimization of resources and maximizing the research output. In order to bridge the gap between Phase II studies focusing on early outcomes and bigger, more expensive Phase III trials (Challenge 6), the use of Phase IIC studies has been advocated. These studies combine early microbiologic endpoints with long patient follow up to capture post-treatment relapses. The CRUSH-TB trial, in development, will likely represent a valuable example of this approach. In this trial, at least two 4-month experimental regimens based on the combination of bedaquiline, moxifloxacin, and pyrazinamide, will be compared to the standard of care for drug-susceptible TB. The primary outcome will be time to sustained culture conversion, but participants will be followed up to 12 months post-randomization, allowing for assessment of relapse-free cure as a secondary outcome.

Phase III TB randomized controlled trials with new designs, such as MAMS trials, Bayesian adaptive trials (Current Trials Example 1), and duration-randomized trials (Current Trials Example 2), are being performed and have the potential to improve efficiency without compromising the generalizability of the results. Other Phase III trials (like BEAT-Tuberculosis and endTB-Q, Current Trials Example 3) pursue the same objective by testing a treatment strategy, which takes into account patient characteristics, such as microbiologic and/or radiologic results. A similar approach will be followed by CURE-TB, a trial under development which aims to combine results from rapid molecular diagnostic tests and factors that have been identified as markers of successful treatment to individualize treatment for classes of patients.

Another promising design that is already being implemented in TB trials is the use of biomarkers to define treatment duration. For instance, PredictTB is combining results from repeated PET/CT scans and microbiological tests to identify

drug-susceptible TB patients that are eligible to receive shorter treatment. Conversely, Predict/EndTB Signature evaluates whether a 22-gene RNA transcriptomic model, which has been shown to accurately predict clinical outcomes for MDR-TB patients [47], may be used to define MDR-TB treatment duration. These approaches will be hopefully instrumental in developing tailored treatment for TB.

In conclusion, a series of TB clinical trial results will be available in the coming years and may revolutionize TB treatment, improving the quality of evidence backing recommendations for both drug-susceptible and -resistant strains. Such improvements are urgently needed to accelerate progress toward the WHO End TB Strategy goals. Political commitment and adequate investments will be required to sustain the momentum and promote innovation in clinical research [48].

References

1. Fox W, Ellard GA, Mitchison DA. Studies on the treatment of tuberculosis undertaken by the British Medical Research Council tuberculosis units, 1946–1986, with relevant subsequent publications. Internaional Journal of Tuberculosis and Lung Disease. 1999;3:S231–79.
2. Fregonese F, Ahuja SD, Akkerman OW, Arakaki-Sanchez D, Ayakaka I, Baghaei P, Bang D, Bastos M, Benedetti A, Bonnet M, Cattamanchi A, Cegielski P, Chien J-Y, Cox H, Dedicoat M, Erkens C, Escalante P, Falzon D, Garcia-Prats AJ, Gegia M, Gillespie SH, Glynn JR, Goldberg S, Griffith D, Jacobson KR, Johnston JC, Jones-López EC, Khan A, Koh W-J, Kritski A, et al. Comparison of different treatments for isoniazid-resistant tuberculosis: an individual patient data meta-analysis. Lancet Respir Med. 2018;6:265–75.
3. Gegia M, Winters N, Benedetti A, van Soolingen D, Menzies D. Treatment of isoniazid-resistant tuberculosis with first-line drugs: a systematic review and meta-analysis. Lancet Infect Dis. 2017;17:223–34.
4. World Health Organization. WHO consolidated guidelines on tuberculosis. Module 4, Module 4, [Internet]. 2020 [cited 2020 Dec 27].Available from: http://www.ncbi.nlm.nih.gov/books/NBK558570/.
5. Guglielmetti L, Huerga H, Khan U, Varaine F. WHO 2019 guidelines on drug-resistant tuberculosis treatment: based on evidence or expert opinion? Eur Respir J. 2020;55:1901935.
6. Gillespie SH, Crook AM, McHugh TD, Mendel CM, Meredith SK, Murray SR, Pappas F, Phillips PPJ, Nunn AJ. Four-month moxifloxacin-based regimens for drug-sensitive tuberculosis. N Engl J Med. 2014;371:1577–87.
7. Merle CS, Fielding K, Sow OB, Gninafon M, Lo MB, Mthiyane T, Odhiambo J, Amukoye E, Bah B, Kassa F, N'Diaye A, Rustomjee R, de Jong BC, Horton J, Perronne C, Sismanidis C, Lapujade O, Olliaro PL, Lienhardt C. A four-month Gatifloxacin-containing regimen for treating tuberculosis. N Engl J Med. 2014;371:1588–98.
8. Jindani A, Harrison TS, Nunn AJ, Phillips PPJ, Churchyard GJ, Charalambous S, Hatherill M, Geldenhuys H, McIlleron HM, Zvada SP, Mungofa S, Shah NA, Zizhou S, Magweta L, Shepherd J, Nyirenda S, van Dijk JH, Clouting HE, Coleman D, Bateson ALE, McHugh TD, Butcher PD, Mitchison DA. High-dose Rifapentine with moxifloxacin for pulmonary tuberculosis. N Engl J Med. 2014;371:1599–608.
9. Warner DF, Mizrahi V. Shortening treatment for tuberculosis — Back to basics. N Engl J Med. 2014;371:1642–3.
10. Imperial MZ, Nahid P, Phillips PPJ, Davies GR, Fielding K, Hanna D, Hermann D, Wallis RS, Johnson JL, Lienhardt C, Savic RM. A patient-level pooled analysis of treatment-shortening regimens for drug-susceptible pulmonary tuberculosis. Nat Med. 2018;24:1708–15.

11. Dorman SE, Nahid P, Kurbatova EV, Phillips PPJ, Bryant K, Dooley KE, Engle M, Goldberg SV, Phan HTT, Hakim J, Johnson JL, Lourens M, Martinson NA, Muzanyi G, Narunsky K, Nerette S, Nguyen NV, Pham TH, Pierre S, Purfield AE, Samaneka W, Savic RM, Sanne I, Scott NA, Shenje J, Sizemore E, Vernon A, Waja Z, Weiner M, Swindells S, et al. Four-month Rifapentine regimens with or without moxifloxacin for tuberculosis - supplementary. N Engl J Med. 2021;384:1705–18.
12. Nunn AJ, Phillips PPJ, Meredith SK, Chiang C-Y, Conradie F, Dalai D, van Deun A, Dat P-T, Lan N, Master I, Mebrahtu T, Meressa D, Moodliar R, Ngubane N, Sanders K, Squire SB, Torrea G, Tsogt B, Rusen ID. A trial of a shorter regimen for rifampin-resistant tuberculosis. N Engl J Med. 2019;380:1201–13.
13. Diacon AH, Patientia R, Krause R, Allen J, Palomino JC, Lounis N, de Beule K. The Diarylquinoline TMC207 for Multidrug-Resistant Tuberculosis. N Engl J Med. 2009;360:9.
14. Diacon AH, Pym A, Grobusch MP, de los Rios JM, Gotuzzo E, Vasilyeva I, Leimane V, Andries K, Bakare N, De Marez T, Haxaire-Theeuwes M, Lounis N, Meyvisch P, De Paepe E, Van Heeswijk RPG, Dannemann B. Multidrug-Resistant Tuberculosis and Culture Conversion with Bedaquiline. N Engl J Med. 2014;371:723–32.
15. Gler MT, Skripconoka V, Sanchez-Garavito E, Xiao H, Cabrera-Rivero JL, Vargas-Vasquez DE, Gao M, Awad M, Park S-K, Shim TS, Suh GY, Danilovits M, Ogata H, Kurve A, Chang J, Suzuki K, Tupasi T, Koh W-J, Seaworth B, Geiter LJ, Wells CD. Delamanid for multidrug-resistant pulmonary tuberculosis. N Engl J Med. 2012;366:2151–60.
16. Skripconoka V, Danilovits M, Pehme L, Tomson T, Skenders G, Kummik T, Cirule A, Leimane V, Kurve A, Levina K, Geiter LJ, Manissero D, Wells CD. Delamanid improves outcomes and reduces mortality in multidrug-resistant tuberculosis. Eur Respir J. 2013;41:1393–400.
17. von Groote-Bidlingmaier F, Patientia R, Sanchez E, Balanag V, Ticona E, Segura P, Cadena E, Yu C, Cirule A, Lizarbe V, Davidaviciene E, Domente L, Variava E, Caoili J, Danilovits M, Bielskiene V, Staples S, Hittel N, Petersen C, Wells C, Hafkin J, Geiter LJ, Gupta R. Efficacy and safety of delamanid in combination with an optimised background regimen for treatment of multidrug-resistant tuberculosis: a multicentre, randomised, double-blind, placebo-controlled, parallel group phase 3 trial. Lancet Respir Med. 2019;7:249–59.
18. Conradie F, Diacon AH, Ngubane N, Howell P, Everitt D, Crook AM, Mendel CM, Egizi E, Moreira J, Timm J, McHugh TD, Wills GH, Bateson A, Hunt R, Van Niekerk C, Li M, Olugbosi M, Spigelman M. Treatment of highly drug-resistant pulmonary tuberculosis. N Engl J Med. 2020;382:893–902.
19. Dooley KE, Rosenkranz SL, Conradie F, Moran L, Hafner R, Groote-Bidlingmaier Von F, Lama JR, Shenje J, Rios JDL, Comins K, Morganroth J, Diacon AH, Cramer YS, Donahue K, Maartens G, Alli O, Gottesman J, Guevara M, Hikuam C, Hovind L, Karlsson M, McClaren J, McIlleron H, Murtaugh W, Rolls B, Shahkolahi A, Stone L, Tegha G, Tenai J, Upton C, et al. QT effects of bedaquiline, delamanid, or both in patients with rifampicin-resistant tuberculosis: a phase 2, open-label, randomised, controlled trial. The Lancet Infectious Diseases [Internet] Elsevier. 2021; [cited 2021 Apr 5]; 0Available from: https://www.thelancet.com/journals/laninf/article/PIIS1473-3099(20)30770-2/abstract
20. Hewison C, Guglielmetti L. Cardiac safety of multidrug-resistant tuberculosis treatment: moving towards individualised monitoring. *The Lancet Infectious Diseases* [Internet] Elsevier; 2021 [cited 2021 May 3]; 0Available from: https://www.thelancet.com/journals/laninf/article/PIIS1473-3099(20)30836-7/abstract.
21. Althunian TA, de Boer A, Klungel OH, Insani WN, Groenwold RHH. Methods of defining the non-inferiority margin in randomized, double-blind controlled trials: a systematic review. *Trials* [Internet] 2017 [cited 2021 May 3]; 18Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5341347/.
22. Nunn AJ, Phillips PPJ, Gillespie SH. Design issues in pivotal drug trials for drug sensitive tuberculosis (TB). Tuberculosis (Edinb). 2008;88(Suppl 1):S85–92.
23. Bratton DJ, Nunn AJ. Alternative approaches to tuberculosis treatment evaluation: the role of pragmatic trials. Int J Tuberc Lung Dis. 2011;15:440–6.

24. Davies GR, Phillips PPJ, Jaki T. Adaptive clinical trials in tuberculosis: applications, challenges and solutions. Int J Tuberc Lung Dis. 2015;19:626–34.
25. Abubakar I, Lipman M. Reducing loss to follow-up during treatment for drug-resistant tuberculosis. European Respiratory Journal [Internet] European Respiratory Society. 2019; [cited 2021 May 3]; 53Available from: https://erj.ersjournals.com/content/53/1/1802268
26. Nissen TN, Rose MV, Kimaro G, Bygbjerg IC, Mfinanga SG, Ravn P. Challenges of loss to follow-up in tuberculosis research. *PLOS ONE* public library of. Science. 2012;7:e40183.
27. Phillips PPJ, Mitnick CD, Neaton JD, Nahid P, Lienhardt C, Nunn AJ. Keeping phase III tuberculosis trials relevant: adapting to a rapidly changing landscape. PLoS Med. 2019;16:e1002767.
28. Guglielmetti L, Low M, McKenna L. Challenges in TB regimen development: preserving evidentiary standards for regulatory decisions and policymaking. Expert Rev Anti-Infect Ther. 2020;18:701–4.
29. Nimmo C, Lipman M, Phillips PPJ, McHugh T, Nunn A, Abubakar I. Shortening treatment of tuberculosis: lessons from fluoroquinolone trials. Lancet Infect Dis. 2015;15:141–3.
30. Nunn AJ, Phillips P, Mitchison D. Timing of relapse in short-course chemotherapy trials for tuberculosis. International journal of tuberculosis and lung disease. 2010;14:241–2.
31. Ginsberg AM, Spigelman M. Challenges in tuberculosis drug research and development. Nat Med. 2007;13:290–4.
32. Bonnett LJ, Ken-Dror G, Koh GCKW, Davies GR. Comparing the efficacy of drug regimens for pulmonary tuberculosis: meta-analysis of endpoints in early-phase clinical trials. Clin Infect Dis. 2017;65:46–54.
33. Global TB Community Advisory Board. Research, Regulatory, and Access Considerations Regarding Pretomanid. http://www.tbonline.info/media/uploads/documents/tb_cab_pretomanid_nda_consideration. 2019.
34. Swindells S, Ramchandani R, Gupta A, Benson CA, Leon-Cruz J, Mwelase N, Jean Juste MA, Lama JR, Valencia J, Omoz-Oarhe A, Supparatpinyo K, Masheto G, Mohapi L, da Silva Escada RO, Mawlana S, Banda P, Severe P, Hakim J, Kanyama C, Langat D, Moran L, Andersen J, Fletcher CV, Nuermberger E, Chaisson RE. One month of Rifapentine plus isoniazid to prevent HIV-related tuberculosis. N Engl J Med. 2019;380:1001–11.
35. Stout JE, Turner NA, Belknap RW, Horsburgh CR, Sterling TR, Phillips PPJ. Optimizing the Design of Latent Tuberculosis Treatment Trials: insights from mathematical modeling. Am J Respir Crit Care Med. 2020;201:598–605.
36. Phillips PPJ, Mendel CM, Burger DA, Crook AM, Crook A, Nunn AJ, Dawson R, Diacon AH, Gillespie SH. Limited role of culture conversion for decision-making in individual patient care and for advancing novel regimens to confirmatory clinical trials. BMC Med. 2016;14:19.
37. Jawahar MS, Banurekha VV, Paramasivan CN, Rahman F, Ramachandran R, Venkatesan P, Balasubramanian R, Selvakumar N, Ponnuraja C, Iliayas AS, Gangadevi NP, Raman B, Baskaran D, Kumar SR, Kumar MM, Mohan V, Ganapathy S, Kumar V, Shanmugam G, Charles N, Sakthivel MR, Jagannath K, Chandrasekar C, Parthasarathy RT, Narayanan PR. Randomized Clinical Trial of Thrice-Weekly 4-Month Moxifloxacin or Gatifloxacin Containing Regimens in the Treatment of New Sputum Positive Pulmonary Tuberculosis Patients. Doherty TM, editor. *PLoS ONE* 2013; 8: e67030.
38. Phillips PPJ, Dooley KE, Gillespie SH, Heinrich N, Stout JE, Nahid P, Diacon AH, Aarnoutse RE, Kibiki GS, Boeree MJ, Hoelscher M. A new trial design to accelerate tuberculosis drug development: the phase IIC selection trial with extended post-treatment follow-up (STEP). BMC Med. 2016;14:51.
39. Horsburgh CR, Shea KM, Phillips P, LaValley M. Randomized clinical trials to identify optimal antibiotic treatment duration. Trials. 2013;14:88.
40. Cellamare M, Ventz S, Baudin E, Mitnick CD, Trippa L. A Bayesian response-adaptive trial in tuberculosis: the *endTB* trial. Clin Trials. 2017;14:17–28.
41. Cellamare M, Milstein M, Ventz S, Baudin E, Trippa L, Mitnick CD. Bayesian adaptive randomization in a clinical trial to identify new regimens for MDR-TB: the endTB trial. Int J Tuberc Lung Dis. 2016;20:8–12.

42. Gao M, Gao J, Du J, Liu Y, Zhang Y, Ma L, Mi F, Li L, Tang S. Trial team. Efficacy of ultra-short course chemotherapy for new smear positive drug susceptible pulmonary tuberculosis: study protocol of a multicenter randomized controlled clinical trial. BMC Infect Dis. 2017;17:435.
43. Guglielmetti L, Varaine F, Mitnick C. Evaluating newly approved drugs for multidrug-resistant tuberculosis (endTB): an adaptive, multi-country randomized controlled trial. Trials. 2021; In press
44. Lee M, Mok J, Kim DK, Shim TS, Koh W-J, Jeon D, Lee T, Lee SH, Kim JS, Park JS, Lee JY, Kim SY, Lee JH, Jo K-W, Jhun BW, Kang YA, Ahn JH, Kim C-K, Shin S, Song T, Shin SJ, Kim YR, Ahn H, Hahn S, Won HJ, Jang JY, Cho SN, Yim J-J. Delamanid, linezolid, levofloxacin, and pyrazinamide for the treatment of patients with fluoroquinolone-sensitive multidrug-resistant tuberculosis (treatment shortening of MDR-TB using existing and new drugs, MDR-END): study protocol for a phase II/III, multicenter, randomized, open-label clinical trial. Trials. 2019;20:57.
45. Moodley R, Godec TR. Short-course treatment for multidrug-resistant tuberculosis: the STREAM trials. Eur Respir Rev. 2016;25:29–35.
46. Lienhardt C, Vernon AA, Cavaleri M, Nambiar S, Nahid P. Development of new TB regimens: harmonizing trial design, product registration requirements, and public health guidance. PLoS Med. 2019;16:e1002915.
47. Heyckendorf J, Marwitz S, Reimann M, Avsar K, DiNardo A, Günther G, Hoelscher M, Ibraim E, Kalsdorf B, Kaufmann SHE, Kontsevaya I, van Leth F, Mandalakas AM, Maurer FP, Müller M, Nitschkowski D, Olaru ID, Popa C, Rachow A, Rolling T, Rybniker J, Salzer HJF, Sanchez-Carballo P, Schuhmann M, Schaub D, Spinu V, Suárez I, Terhalle E, Unnewehr M, Weiner J, et al. Prediction of anti-tuberculosis treatment duration based on a 22-gene transcriptomic model. Eur Respir J. 2021;58
48. Nyang'wa B-T, LaHood A, Mitnick CD, Guglielmetti L. TB research requires strong protections, innovation, and increased funding in response to COVID-19. Trials. 2021;22:1–3.

Chapter 13
Contributions of Japanese Scholars to Advances in Clinical TB Management: In TB World, What Kind of Achievements Are Accomplished by Japanese Researchers?

Isano Hase and Takefumi Saito

Abstract Since the discovery of *Mycobacterium tuberculosis* by Robert Koch in 1882, researchers worldwide have focused on preventing, diagnosing, and treating tuberculosis (TB). Owing to these efforts, what was once considered a nation-ruining disease has now become treatable. Although Japanese researchers have played a major role in exploring TB, their contributions have not always been appropriately recognized internationally. This chapter details the four major contributions made by Japanese researchers toward the prevention of TB as well as elucidation of its pathology and treatment.

Keywords BCG Tokyo 172 · Theory of onset following primary infection · Delayed-type hypersensitivity · Kanamycin

1 Introduction

Globally, Japanese researchers have made immense contributions toward better understanding of TB. However, many of their research achievements are communicated in forms that are inaccessible to those outside Japan, with reference to oral presentations as well as research articles and books published in Japanese. As a result, Japan's valuable contributions to TB research, including discoveries that

I. Hase (✉) · T. Saito
Department of Respiratory Medicine, National Hospital Organization Ibarakihigashi Hospital, Naka-gun, Ibaraki, Japan

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could have been considered the world's firsts, have gone largely unnoticed by the international community. The objective of this chapter is to introduce the great achievements made by Japanese researchers that were ahead of their time.

2 Bacille Calmette-Guérin (BCG) Tokyo 172: Substrain of Pasteur BCG: Since 1924

In 1921, Albert Calmette and Camille Guérin of the Pasteur Institute developed the BCG vaccine, the first live vaccine against TB. Although the hydrophobic nature of *Mycobacterium tuberculosis* and its tendency to aggregate posed challenges to its culturing, Calmette and Guérin discovered that adding ox bile to the culture medium prevented *M. tuberculosis* aggregation. They used glycerin, ox bile, and potato medium as a culture medium ideal for detoxification. Using these methods over the course of 13 years and 231 passages, they succeeded in attenuating *Mycobacterium bovis*, a virulent strain of TB in cattle. Calmette frequently described BCG as "Bacille bilié Calmette-Guérin" or "Bacille bilies," suggesting the importance of bile in the development of BCG. The vaccine was first administered to humans in 1921 and was later adopted worldwide. Japanese researchers have played a major role in the development of technological innovations associated with the spread and utilization of BCG.

2.1 BCG Tokyo 172: Since 1924

The history of BCG in Japan began in 1924, when Kiyoshi Shiga of the Kitasato Institute received BCG from Calmette at the Pasteur Institute and brought it back to Japan. The Japanese strain of BCG was developed through repeated passages in a special medium containing ox bile in faithful adherence to Calmette's method. Although early BCG strains were initially subcultured as liquid vaccines, a freeze-dried seed lot was prepared from the Tokyo 172 strain in 1961. In 1965, the World Health Organization (WHO) selected the BCG Tokyo 172 strain as the first international reference for the freeze-dried BCG vaccine.

Although the original BCG strain developed at the Pasteur Institute was distributed to many countries, repeated passages using variations of Calmette's method at different facilities produced diverse sub-strains with different biological properties. Although BCG Tokyo 172 is less virulent than other BCG sub-strains, it is superior in terms of aerobic plate counts per unit weight of vaccine (i.e., high titer) and thermal stability [1, 2]. A multiplex polymerase chain reaction (PCR)-based investigation of genes in regions of difference (RD) of BCG sub-strains revealed that although RD2 was absent in BCG sub-strains sent to various countries by the Pasteur Institute from 1926 onward (late strains; BCG Danish 1331, etc.), it was preserved in BCG

strains sent to various countries prior to 1926 (early strains; BCG Japan, BCG Russia, etc.) (Figure 13.1) [3]. Reportedly, BCGs produced using Tokyo 172 and other early strains induced the release of inflammatory cytokines much more vigorously than the late strains [4, 5]. This suggested that BCG Tokyo 172, which is produced in a bile-containing medium in a similar manner to that of the original BCG and approximates it closely, was highly effective and safe.

In 2003, the WHO enacted a policy for the determination of multiple international reference preparation candidates for each BCG substrain used worldwide. These decisions were based on factors such as colony-forming units (CFUs), ATP levels, and multiplex PCR. Based on these results, the WHO Expert Committee on Biological Standardization established the following international reference reagents: Tokyo 172–1, a Tokyo 172-derived seed lot; Danish 1331, BCG Russia (BCG-I), and the Brazilian Moreau-RJ.

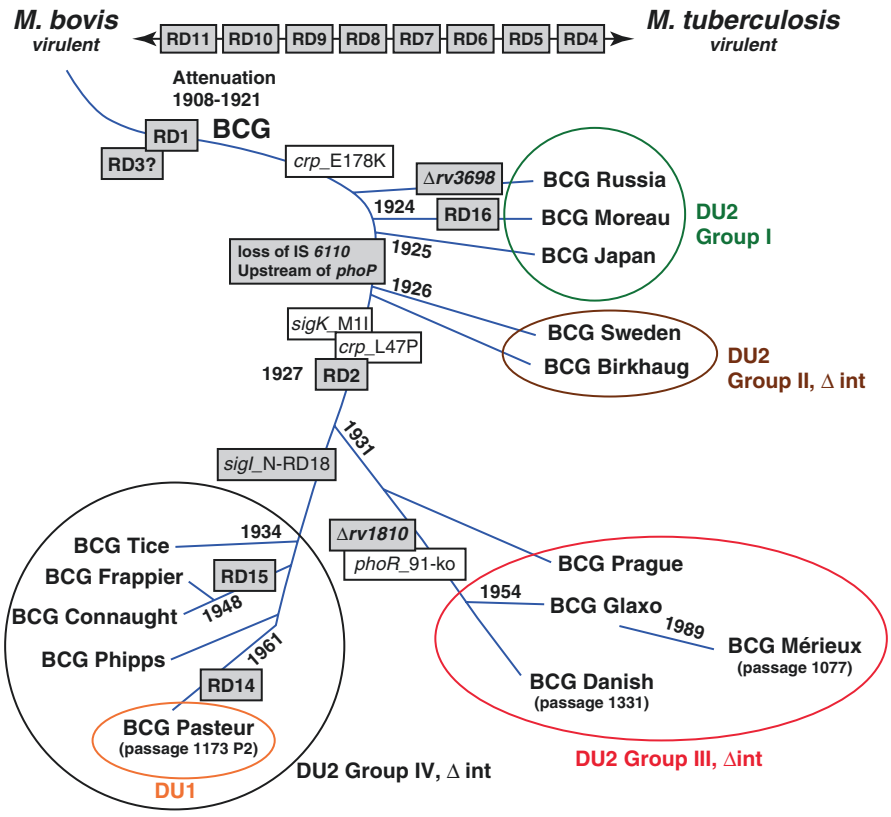

Fig. 13.1 Refined genealogy of BCG vaccines [3]. The process by which each BCG substrain was derived from *Mycobacterium bovis* based on the loss of regions of differences (RDs). Sub-strains of BCG are divided into early strains (supplied by the Pasteur Institute prior to the deletion of RD2 in 1927) and late strains (supplied from 1930 onward)

2.2 The Development of Highly Heat-Resistant, Long-Life Dried Vaccines: Since 1943

The original BCG vaccine was a live, liquid vaccine with a shelf life of only 1–2 weeks, which hindered its transport to remote areas and pre-usage experiments on the efficacy and safety of the vaccine. As a resolution to this issue, Japanese researchers developed freeze-dried vaccines. This process is broadly divided into the following: (1) extending shelf life by freeze-drying and (2) long-term storage at room temperature.

In 1943, an army physician named Takeo Hayashi discovered a freeze-drying method involving the use of 5% lactose as the medium, which enabled CFUs to be stored for 5 months or more and still be effective against TB [6]. Yoji Obayashi of the Japan Anti-Tuberculosis Association and colleagues succeeded in mass-producing freeze-dried BCG vaccines using a chamber-type desiccator with sucrose as the medium [7]. This development resulted in a national policy that made all individuals with a negative tuberculin reaction eligible for BCG inoculation from 1948 onward. In addition, Tatsuichiro Hashimoto of Tsukuba University discovered that BCG strains cultured for 7–9 days developed a high degree of resistance to freeze drying, with a resultant increase in the number of CFUs in vaccines and improved titers [8].

Early freeze-dried BCG vaccines need to be preserved at low temperatures (≤5 °C). However, Yoji Obayashi and Chujo Cho determined that the use of 1% sodium glutamate as a medium enabled preservation of BCG vaccines at 37 °C for 6–8 months [9–11]. This discovery led to the widespread use of BCG, particularly in developing tropical countries without cold chains. In addition, while freeze-dried BCG vaccines had replaced live vaccines worldwide, the adoption of sodium glutamate as a medium had enabled the production of vaccines with improved heat resistance. Although the fact that this Japanese technology was made available without being patented remains largely unrecognized, this bold decision has contributed immensely to TB prevention around the world.

2.3 Devising a Route for Administration: Percutaneous Vaccination with a Nine-Needle Stamp: 1961

The very first BCG administered to a human was orally to a newborn baby of a woman with severe TB. The mother died shortly after giving birth, and the baby was raised by her grandmother, who also had TB. However, the baby did not develop TB and grew up without experiencing any major side effects. Since the efficacy of orally administered BCG diminishes after the neonatal period, subcutaneous injections were attempted. In 1929, the first subcutaneous BCG injection was administered to an infant in Japan. However, following inoculation, the infant became prone to local ulceration and abscesses. Therefore, in 1941, intradermal injections, which

only generated a mild local response but led to high rates of tuberculin reactions, were adopted as the standard in Japan. Unfortunately, the administration of intradermal injections was beset with technical difficulties as these injections were frequently associated with side effects such as ulcerations and abscesses that manifested due to needles accidentally slipping under the skin [6]. Therefore, percutaneous injections were explored to avoid these side effects. The multiple puncture method, which uses a special apparatus, was reported outside Japan by Rosenthal [12] and Birkhaug [13]. In Japan, Gorosaku Kuchiki and colleagues at the BCG Laboratory developed a method of injection that utilizes a cylinder implanted with nine fine needles [6]. This method was highly acclaimed for its ability to reduce the side effects at the injection site and enable a simple and reliable percutaneous injection for infants, for whom BCG injection was intended. In 1967, Japan switched from intradermal BCG injections to percutaneous ones [6, 14]. Intradermal injection continues to be the norm elsewhere in the world [15, 16]. From 2001 to 2004, the University of Cape Town conducted a randomized trial that compared percutaneous and intradermal injections of the Tokyo 172 BCG vaccine, the results of which indicated that percutaneous injection with a nine-needle stamp was equivalent to intradermal injection in terms of efficacy and safety [17].

The WHO recommends both intradermal and percutaneous BCG injections [18]. Percutaneous BCG injection administered via the Japanese nine-needle stamp requires a concentration of 80 mg/mL, which is 160 times that required for an intradermal injection (0.5 mg/mL), and is therefore contraindicated for intradermal injection. Thus, it is stipulated that percutaneous BCG vaccines should be clearly labeled as being for percutaneous injection only. BCG inoculation effectively prevents severe pediatric TB such as tuberculous meningitis and miliary TB. Pediatric TB in Japan declined as BCG inoculation of infants and toddlers became common. The WHO recommends that newborns be inoculated with BCG as early as possible. In addition, BCG inoculations are conducted as part of the Expanded Program on Immunization, primarily for children in developing countries. Based on the Expanded Program on Immunization, the United Nations Children's Emergency Fund (UNICEF) procures BCG from WHO licensed vaccine manufacturers and distributes it to developing countries. The Japan BCG Laboratory has a WHO issued license to manufacture BCG vaccines for the UNICEF and exports intradermal vaccines for the UNICEF.

3 The Theory of TB Onset Following Primary Infection: 1929

Theories regarding the onset of TB differ according to time and place and have changed with progress in various research areas, including pathology. The theory of onset following reinfection was once the dominant theory for the mechanism underlying the onset of adult TB in the West. At that time, despite a high rate of 94%

positive tuberculin reactions in children 11–14 years in Europe [19], TB occurred frequently in adults. In addition, primary complexes in adults are often calcified and healed. These facts led to the concept that TB infections remain latent in children and develop in adulthood due to exogenous reinfection.

Japan, in contrast, espoused the theory of onset following primary infection, which holds that TB develops from the very first infection of *M. tuberculosis* in one's lifetime. Japanese researchers conducted several studies that corroborated and cemented this theory. The modern view holds that TB occurs in 10% of people who have experienced primary TB infection at some point in their lives; half of these people may develop TB as a primary disease 2–3 years following infection, while the other half may develop TB due to reactivation of a latent foci [20]. The theory of onset following primary infection forms the basis of the modern theory of TB onset.

3.1 Caseous Foci in Adolescent Patients with TB: 1929

The prevalence of TB in Japan peaked in the 1910s, which was later than in the West. During this period, adolescent to early adult TB patients developed pulmonary TB and extrapulmonary TB from primary infections and frequently died. In 1929, Harumichi Oka of the University of Tokyo autopsied a young marine who had developed miliary TB after a long-distance swim and died. His foci resembled those of a primary complex of TB. Based on this finding, Oka suspected that disease onset had occurred immediately following primary infection. Comparisons of primary complexes of TB in autopsied lungs of patients from different age groups revealed that in adults, caseous foci were infrequent and calcification was common, whereas young patients frequently displayed caseous foci (Fig. 13.2). Caseous foci indicate

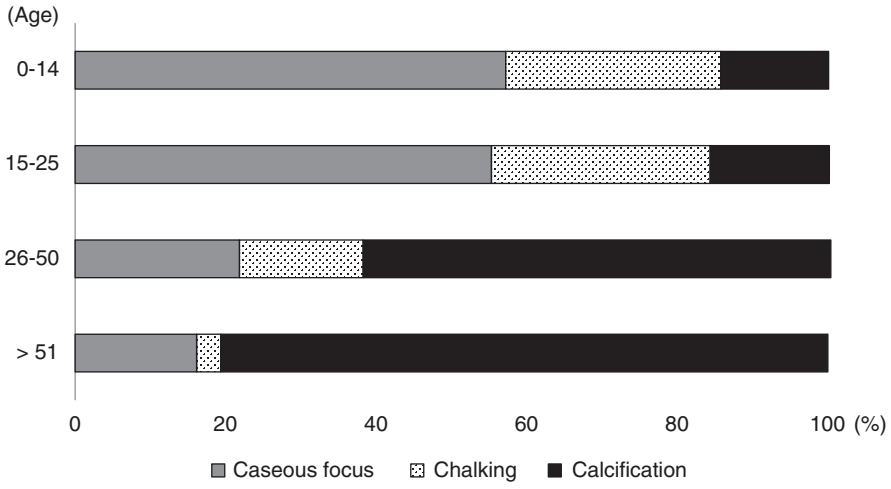

Fig. 13.2 Pathological findings in primary complexes by age group in Tokyo (modified from [21])

a very recent TB infection. Therefore, in Japan at the time, TB in adults was believed to develop frequently from a primary infection [21, 22].

3.2 The Relationship Between Tuberculin Conversions and TB Onset: 1931

Although the presence of a TB infection may currently be assessed via an interferon-gamma release assay, the only available test at that time was the tuberculin reaction. At that time, many young men drafted into the military developed tuberculous pleurisy. Army physician Yoshio Kobayashi regularly performed tuberculin skin tests for members of the navy. He utilized radiographs taken before and after tuberculin conversion to assess the occurrence of pulmonary TB and pleurisy in tuberculin converters. These assessments revealed the following: (i) 30–40% of military personnel had a negative reaction; (ii) regular tuberculin skin tests that yielded negative reactions in military personnel would sometimes present positive reactions later; and (iii) a large percentage of tuberculin converters developed pulmonary TB or tuberculous pleurisy 6–12 months later. These findings proved that many adults are never infected with TB. When TB infections occur in adulthood, TB often develops shortly after infection [23]. Kobayashi coined the term "tuberculin converter" [22].

3.3 The Relationship Between Infections and TB Onset as Revealed by X-Rays: Since 1941

In 1936, Yoshihiko Koga of the Tohoku University and Brazilian physician Manoel de Abreu independently developed the world's first device, which enabled efficient TB screening via indirect imaging of the chest with X-rays. Yasuyuki Chiba and Masao Tokorozawa of the former Japan National Railways Central Health Control (currently the JR East Health Promotion Center) conducted TB screening for approximately 50,000 railway employees. Target subjects were who present no positive tuberculin reactions or X-ray-based findings of TB during the initial round of testing. The radiography performed 1 year later revealed that TB had developed in 0.5% of these employees. These tuberculin response-negative employees were then retested for tuberculin every 3–6 months. Following tuberculin conversion, the employees were screened for another 30 years using radiography. Analysis of the tuberculin converter cohort who had not been inoculated with BCG indicated that the rate of TB onset was highest (16%) within 1 year of tuberculin conversion. For the next 2–15 years, this figure decreased to roughly 1% and subsequently to ≤0.3% [24]. This valuable study corroborated the natural history of TB, particularly the theory of onset following primary infection.

4 Delayed-Type Hypersensitivity: 1954

TB-induced lung cavities occur more frequently and have thicker walls than the cavities caused by other bacterial infections. The treatments in use during this time, such as streptomycin (SM), isoniazid, and para-amino-salicylic acid (PAS), are unable to cure tuberculous cavities, leading to persistent excretion of bacilli and the emergence of resistant bacteria. Yuichi Yamamura et al. succeeded in forming cavities in rabbits in the same manner as that formed in humans, thus demonstrating that tuberculous cavitation is due to an over response by the host to *M. tuberculosis*. It was surprising to discover that the pathogenesis of disease was mediated by an allergic response. A research group headed by Yamamura conducted basic experiments on tuberculous cavitation, which is introduced in the following section.

4.1 Cavitation Experiments: 1954 [25–27]

Studying tuberculous cavities requires these cavities to be experimentally created in animals. Direct injection of live TB bacilli suspended in saline solution into lungs created acute exudative foci but failed to produce caseous foci or cavities. This is believed to be due to bacterial composition not accumulating locally in the lung. However, rabbits developed cavities when exposed to live airborne TB bacilli. Ratcliffe and Wells [28] divided rabbits into two groups, where one group was infected with a small amount of *M. tuberculosis* by inhalation in advance (pre-sensitized group) and the other was not (control group); both were reinfected with a large amount of *M. tuberculosis* via inhalation. Cavities were formed in some rabbits of the pre-sensitized group 2–3 months later, which corresponded to when ≥5 weeks had passed between sensitization and the second infection experiment. In contrast, all rabbits in the control group died within 4 weeks. Although this experiment demonstrated that pre-sensitization is crucial to cavitation, it should be noted that airborne exposure requires a special device, and cavitation did not always occur, and cavities required a long time to form.

In 1954, a research group at the National Sanatorium Toneyama Hospital (currently the Osaka Toneyama Medical Center) including Yuichi Yamamura and others discovered a simpler, more successful, and more reliable method of inducing cavitation [26, 27]. Live bacilli of the Ravenel strain of *M. bovis* (human and bovine) and heat-killed bacilli were injected transthoracically into the lungs of rabbits. Bacteria were suspended in a liquid paraffin/anhydrous lanolin compound (hereafter, “oil extract”) to ensure local retention of bacterial composition in the lung. A group of rabbits that were pre-sensitized with a subcutaneous injection of heat-killed bacilli was compared with a group of nonsensitized rabbits (control group) (Table 13.1).

When live bacilli (1 mg) were injected into the lung along with an oil extract, all sensitized rabbits demonstrated cavitation in 1 month, in a manner similar to that seen in TB in humans. On the other hand, cavities were observed in only half of the rabbits

Table 13.1 Tuberculous cavitation experiment by Yamamura et al., based on [25]

Tubercle Bacilli	Rabbits	Dose of Bacilli for pulmonary injection (mg)	Time between pulmonary injection and autopsy (days)	Number of rabbits tested	Number of rabbits that produced cavities
Living	Sensitized	1	30	4	4
		1	60	4	4
		1	70–350	7	7
		0.5	30–60	4	2
		0.25	30–60	4	1
		0.05	30–60	4	0
	Nonsensitized	1	30–60	5	2
Heat-killed	Sensitized	1	30	14	6
		1	60	7	6
		10	30	6	5
		10	60	1	1
		1	30	3	0
	Nonsensitized	10	30	3	0

at 1 month in the control group. Surprisingly, 43% of the sensitized rabbits whose lungs were injected with heat-killed bacilli (1 mg) demonstrated cavitation at 30 days. Later, it was found that cavitation eventually occurred even in nonsensitized rabbits when the dose of injected bacilli (including heat-killed bacilli) was subsequently increased. Histologically, compared with nonsensitized rabbits, sensitized rabbits demonstrated more localized and smaller lesions, earlier and more pronounced caseating necrosis, and earlier and more widespread layers of granulation tissue.

This experiment demonstrated that tuberculous cavities, which were believed to be formed when *M. tuberculosis* directly destroys lung tissue, could be induced even by killed bacilli if the amount of TB bacterial composition is high enough. The experiment also determined that cavities are highly likely to form early in the event of pre-sensitization to *M. tuberculosis*. In addition, the mechanism of cavitation was found to be cellular immune response to *M. tuberculosis*, namely delayed-type hypersensitivity (DTH).

4.2 Cavity Prevention Experiments: 1968, 1974 [29, 30]

The above-stated experiment suggested that inhibiting DTH prevents cavitation, prompting further research. The first method that attempted to test this used desensitization with frequent tuberculin injections [29]. Rabbit lungs were first injected transthoracically with heat-killed tubercle bacilli. The tuberculin group was then subjected to intravenous injections of a tuberculin-active peptide three times a week, while the control group received no further intervention. The tuberculin group demonstrated no cavitation and had negative tuberculin reactions, whereas the control

group demonstrated cavitation in 50% of cases and had positive tuberculin reactions. The second method that attempted to prevent cavitation used the immunosuppressant azathioprine [30]. Following transthoracic injection of rabbit lungs with heat-killed tubercle bacilli, groups of rabbits that received immunosuppressants were compared with a control group that had not received immunosuppressants. Rabbits that received daily injections of azathioprine did not demonstrate cavities 30 days later and had negative tuberculin reactions, whereas half of the rabbits that did not receive immunosuppressants displayed cavities. The same experiment was conducted using live tubercle bacilli. For this experiment, rabbits were divided into three groups: azathioprine + chemotherapy (SM + isoniazid); chemotherapy only; and azathioprine only. Cavitation was prevented by azathioprine + chemotherapy, but not by chemotherapy alone or azathioprine alone. Azathioprine combined with chemotherapy conceivably prevented cavitation by simultaneously inhibiting tubercle propagation as well as immune response. The reason that cavitation was not prevented in the other two groups could be due to the fact that chemotherapy alone inhibits tubercle bacilli propagation leaving a certain volume of antigens and azathioprine alone fails to prevent tubercle bacilli propagation leaving a large volume of antigens.

4.3 The Search for Cavity-Forming Antigens: 1977 [31]

The finding that cavities are formed by killed tubercle bacilli prompted attempts to retrieve the antigenic substances associated with cavitation caused by these bacilli. Masatami Yamaguchi et al. reported that lipoproteins extracted from heat-killed tubercle bacilli and injected into the lungs of nonsensitized rabbits reliably induced cavity formation approximately 1 month later [32]. Hideo Maeda et al. separated these lipoproteins into lipid and protein fractions via Sephadex LH-20 column chromatography. Although neither the lipid fraction nor the protein fraction caused cavities when injected in isolation into rabbit lungs, cavities were formed when the two fractions were recombined and injected [31]. However, this form of cavitation resulted in thinner walls and a lower rate of formation than cavitation caused by lipoproteins. These findings suggest that unaltered lipoproteins with lipid and protein fractions bound to each other are more effective than recombination of separate fractions. These results demonstrated that the antigenic substances that form cavities are tubercle bacilli-derived lipoproteins and that the presence of lipids with incredibly powerful adjuvant action unique to *M. tuberculosis* formed the origin of sensitization.

5 Kanamycin (KM): 1957

The aminoglycoside bacterial antibiotic, KM, developed in Japan, is the first antibiotic in Japan to have garnered high international acclaim. In addition, an investigation of the mechanisms underlying KM resistance has led to the discovery of various highly effective antibiotics that are still being used in clinical practice.

5.1 The Discovery of KM and Bacteria That Produce it: 1957

KM is a novel antibiotic that was discovered in 1957 by Hamao Umezawa and Yoshiro Okami at the former National Institute of Health (currently the National Institute of Infectious Diseases) [33]. In 1943, S. Waksman derived SM, the world's first antitubercular drug, from the actinomycete *Streptomyces griseus*. SM and PAS, which were discovered in 1946, yielded revolutionary improvements in therapeutic effects. However, TB came to be considered as a disease that was difficult to cure completely with drugs due to emergence of bacterial resistance. To identify new agents that would be effective against *M. tuberculosis*, Umezawa et al. isolated and cultured actinomycetes from soil samples collected throughout Japan and investigated their antimicrobial activity against acid-fast bacilli. An ideal antitubercular agent was considered to be a water-soluble, basic substance that was effective in vivo against *M. tuberculosis* but without persistent toxicity. In 1955, Umezawa, along with Yoshiro Okami, discovered an antibiotic produced by the novel strain K2j, which was isolated from soil samples from the Utsukushigahara Highlands in Nagano Prefecture. Using a cation exchange resin, they extracted and refined an antibiotic that displayed antimicrobial activity against *Escherichia coli* and SM-resistant bacteria [34]. The colonies of strain K2j that formed on agar medium were gold colored (in Japanese, *kana*), which led to the name KM. Strain K2j was then named *Streptomyces kanamyceticus* [33, 34]. The chemical formula of KM is $C_{18}H_{36}N_4O_{11}$, and its chemical structure consists of 6-amino-glucose and 3-amino-glucose on either side of 2-deoxystreptamine (Fig. 13.3) [35].

5.2 KM Is Approved for Therapeutic Use the Year after Its Discovery: 1958

KM was found to be effective against *Streptococcus pneumoniae*, *Staphylococcus aureus*, and *Salmonella typhi* in mice. In addition, animal experiments demonstrated that KM was almost completely unabsorbed when ingested orally and effective when injected intramuscularly. These experiments also indicated that the metabolic pathway of KM was associated with the kidneys. In 1957, Tokuji Ichikawa and colleagues at the University of Tokyo began administering KM to humans. KM was

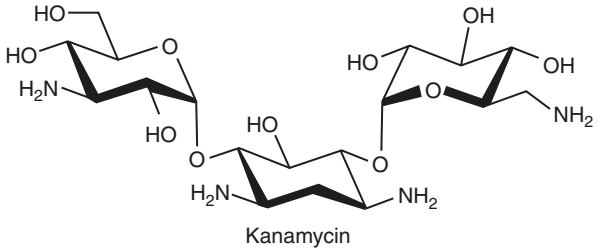

Fig. 13.3 Structural formula of Kanamycin [35]

found to be clinically effective against *S. aureus* and Gram-negative bacterial infections. Therefore, the production and sale of KM in Japan began in 1958. That same year, KM earned high acclaim in forms, such as the special symposium held by the New York Academy of Science. In 1959, KM was exported to the United States and subsequently adopted for widespread clinical use in Europe. Outside Japan, KM was initially used as a therapeutic agent for resistant staphylococcal infections [36] and has saved the lives of many severely ill patients [37]. Later, KM was found to be effective against various resistant bacteria. Thus, KM has been used in many countries [34], making it the first Japanese pharmaceutical product to gain high international acclaim.

5.3 Research on the Mechanism Underlying KM Resistance and the Resultant Novel Antibiotics

Worldwide use of SM, developed in 1943, led to the evolution of resistant bacteria which posed a serious challenge. In 1957, Ken Yanagisawa, Naoyuki Sato, and colleagues at the National Institute of Health reported that KM was effective against SM- and PAS-resistant TB [38]. Intriguingly, KM-resistant strains were also resistant to SM, a phenomenon which was termed one-way cross-resistance between SM and KM [39, 40]. This suggested that it was appropriate to administer SM for TB, before administering KM.

As KM is widely used worldwide, reports of KM-resistant bacteria have emerged. Umezawa et al. elucidated the mechanism underlying resistance to aminoglycoside antibiotics. These studies led to the creation of antibiotics effective against resistant bacteria by chemically removing or exchanging structural sites of KM that were susceptible to modification by bacterial enzymes. In this manner, dibekacin, arbekacin, and amikacin (AMK) were developed from KM in Japan [41]. Currently, AMK is being used to treat drug-resistant TB and nontuberculous mycobacterial infections.

6 Conclusion

Multifaceted research studies conducted on TB in Japan have covered the prophylactics (BCG Tokyo strains and freeze-dried vaccines), TB-associated pathology (the theory of TB onset following primary infection, delayed-type hypersensitivity), and therapeutic agents (KM). Thus, it may be fair to declare that research studies pioneered in Japan, together with research conducted in other countries, have helped form the basis of current TB research. Therefore, this chapter may be considered as an attempt to express our respect and gratitude to the great Japanese researchers of the past.

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References

1. Ho MM, Markey K, Rigsby P, Hockley J, Corbel MJ. Report of an international collaborative study to establish the first WHO reference reagents for BCG vaccines of three different substrains. Vaccine. 2011;29:512–8. https://doi.org/10.1016/j.vaccine.2010.10.066.
2. Sher NA, Chaparas SD, Pearson J, Chirigos M. Virulence of six strains of Mycobacterium bovis (BCG) in mice. Infect Immun. 1973;8:736–42. https://doi.org/10.1128/IAI.8.5.736-742.1973.
3. Brosch R, Gordon SV, Garnier T, Eiglmeier K, Frigui W, Valenti P, et al. Genome plasticity of BCG and impact on vaccine efficacy. Proc Natl Acad Sci U S A. 2007;104:5596–601. https://doi.org/10.1073/pnas.0700869104.
4. Davids V, Hanekom WA, Mansoor N, Gamieldien H, Gelderbloem SJ, Hawkridge A, et al. The effect of bacille Calmette-Guérin vaccine strain and route of administration on induced immune responses in vaccinated infants. J Infect Dis. 2006;193:531–6. https://doi.org/10.1086/499825.
5. Hayashi D, Takii T, Fujiwara N, Fujita Y, Yano I, Yamamoto S, et al. Comparable studies of immunostimulating activities in vitro among Mycobacterium bovis bacillus Calmette-Guérin (BCG) substrains. FEMS Immunol Med Microbiol. 2009;56:116–28. https://doi.org/10.1111/j.1574-695X.2009.00559.x.
6. Toida I. BCG, a tuberculosis vaccine--Japanese contribution. (in Japanese). Kekkaku: [Tuberculosis]. 2011;86:603–6.
7. Obayashi Y, Organization WH. Dried BCG vaccine/Yoji Obayashi. World Health Organization monograph series; no 28. Geneva: World Health Organization; 1955.
8. Hashimoto T. Tuberculosis prevention by BCG vaccine. (in Japanese). Kekkaku: [Tuberculosis]. 1982;57:329–34.
9. Cho C, Iwasaki T, Kawasaki J, Obayashi Y. Effect of storage at 37 degrees C on immunizing power of dried BCG vaccine. Bull World Health Organ. 1956;14:671–80.
10. Cho C, Obayashi Y. Effect of adjuvant on preservability of dried BCG vaccine at 37 degrees C. Bull World Health Organ. 1956;14:657–69.
11. Obayashi Y, Kawasaki J, Yoshioka T, Shimao T, Noguchi T. Effect of preservation on the viability and allergenic potency of dried BCG vaccine. Bull World Health Organ. 1956;14:529–42.
12. Rosenthal SR. The multiple puncture method of BCG vaccination. Am Rev Tuberc. 1939;39:128–34.
13. Birkhaug K. A detachable spring-actuated multiple puncture apparatus for BCG vaccination. Am J Clin Pathol. 1949;19:492–5. https://doi.org/10.1093/ajcp/19.5_ts.492.
14. Yamamoto S, Yamamoto T. Historical review of BCG vaccine in Japan. Jpn J Infect Dis. 2007;60:331–6.
15. Toida I. BCG vaccination in the world. (in Japanese). Kekkaku: [Tuberculosis]. 2000;75:1–7.
16. Bricks LF. Percutaneous or intradermal BCG vaccine? J Pediatr (Rio J). 2004;80:93–8.
17. Hawkridge A, Hatherill M, Little F, Goetz MA, Barker L, Mahomed H, et al. Efficacy of percutaneous versus intradermal BCG in the prevention of tuberculosis in south African infants: randomised trial. BMJ. 2008;337:a2052. https://doi.org/10.1136/bmj.a2052.
18. Ho MM, Southern J, Kang HN, Knezevic I. WHO informal consultation on standardization and evaluation of BCG vaccines Geneva, Switzerland 22-23 September 2009. Vaccine. 2010;28:6945–50. https://doi.org/10.1016/j.vaccine.2010.07.086.
19. Hamburger F. The incidence of tuberculosis in childhood. Br Med J. 1910;2:76–7. https://doi.org/10.1136/bmj.2.2584.76-a.
20. Schlossberg D. Tuberculosis and nontuberculous mycobacterial infections. 6th ed. Washington, DC: ASM Press; 2011.
21. Oka H. Tuberculosis prevention problems and their systems. (in Japanese). Kekkaku: [Tuberculosis]. 1932;10:39–51.
22. Shimao T. Theory of the onset of initial contraction of tuberculosis. (in Japanese). Kekkaku: [Tuberculosis]. 2011;86:583–6.
23. Kobayashi Y. Tuberculin Allergy and Pleurisy. (in Japanese). Kekkaku: [Tuberculosis]. 1931;9:1291–395.

24. Chiba Y. Significance of endogenous reactivation. 30 years' observation of subjects whose tuberculin test reaction has changed. Bull Int Union Tuberc. 1974;49:321–4.
25. Yamamura Y. The pathogenesis of tuberculous cavities. Bibl Tuberc. 1958;13:13–37.
26. Yamamura Y, Yasaka S, Yamaguchi M, Endo K, Iwakura H, Nakamura S, et al. Experimental formation of the tuberculous cavity in the rabbit's lung; experimental study on the tuberculous allergy. I. (in Japanese). Kekkaku: [Tuberculosis]. 1954;29:143–6; English abstract, 53–4.
27. Yamamura Y, Yasaka S, Nakamura S, Ogawa Y, Yamaguchi M, Endo K, et al. Experimental studies on the tuberculous allergy. III. Experimental formation of the tuberculous cavity in the rabbit's lung by killed tubercle bacillus. (in Japanese). Kekkaku: [Tuberculosis]. 1954;29:361–7; English abstract, 76–7.
28. Ratcliffe HL, Wells WF. Tuberculosis of rabbits induced by droplet nuclei infection; response to reinfection. J Exp Med. 1948;87:585–94. https://doi.org/10.1084/jem.87.6.585.
29. Yamamura Y, Ogawa Y, Maeda H. Prevention of tuberculous cavity formation by desensitization with tuberculin-active peptide. Am Rev Respir Dis. 1974;109:594–601. https://doi.org/10.1164/arrd.1974.109.6.594.
30. Yamamura Y, Ogawa Y, Yamagata H. Prevention of tuberculous cavity formation by immunosuppressive drugs. Am Rev Respir Dis. 1968;98:720–3. https://doi.org/10.1164/arrd.1968.98.4.720.
31. Maeda H, Yamamura Y, Ogawa Y, Maeda J. Mycobacterial antigens relating to experimental pulmonary cavity formation. Am Rev Respir Dis. 1977;115:617–23. https://doi.org/10.1164/arrd.1977.115.4.617.
32. Yamaguchi M, Ogawa Y, Endo K, Takeuchi H, Nakamura S, Yasaka S, et al. Experimental formation of tuberculous cavity in rabbit lung. V. Experimental production of tuberculous cavity by chemical constituents of tubercle bacilli. (in Japanese). Kekkaku: [Tuberculosis]. 1958;33:12–6; English abstract 48.
33. Umezawa H, Ueda M, Maeda K, Yagishita K, Kondo S, Okami Y, et al. Production and isolation of a new antibiotic: kanamycin. J Antibiot (Tokyo). 1957;10:181–8.
34. Umezawa H. Kanamycin: its discovery. Ann N Y Acad Sci. 1958;76:20–6. https://doi.org/10.1111/j.1749-6632.1958.tb54688.x.
35. Hotta K, Yagisawa M, Yamamoto H, Kondo S. The way of Dr. Hamao Umezawa from kanamycin discovery to the Japan academy prize and the order of cultural merit. (in Japanese). Jpn J Antibiot. 2017;70:201–10.
36. Finland M. Kanamycin Lancet. 1958;2:209–11. https://doi.org/10.1016/s0140-6736(58)91545-9.
37. Bunn PA, Baltch A. Clinical experiences with kanamycin. N Engl J Med. 1958;259:659–62. https://doi.org/10.1056/NEJM195810022591401.
38. Yanagisawa K, Sato N. Studies on kanamycin, a new antibiotic against tubercle bacilli. I. Effect on virulent tubercle bacilli in vitro and in mice. J Antibiot (Tokyo). 1957;10:233–5.
39. Tsukamura M. Drug-resistance test for kanamycin and cross resistance problem in mycobacterium tuberculosis with special reference to kanamycin, streptomycin, and viomycin. J Antibiot (Tokyo). 1959;12:189–91.
40. Tsukamura M, Yamamoto M, Hayashi M, Torii F, Noda Y. Studies on cross resistance in Mycobacterium tuberculosis. II. (in Japanese). Kekkaku: [Tuberculosis]. 1961;36:233–6.
41. Kawaguchi H, Naito T, Nakagawa S, Fujisawa KI. BB-K 8, a new semisynthetic aminoglycoside antibiotic. J Antibiot (Tokyo). 1972;25:695–708. https://doi.org/10.7164/antibiotics.25.695.

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Figure 3 of chapter 2 was initially published with errors. This has been corrected.

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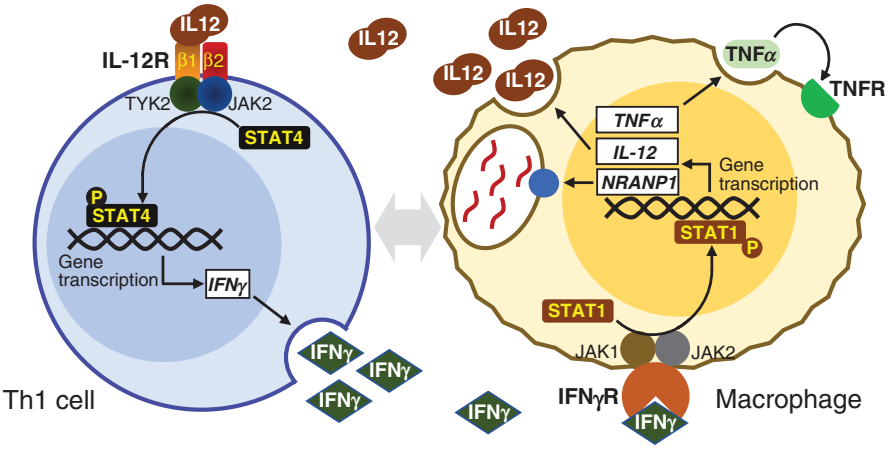

Fig. 2.3 Antimycobacterial immunity induced by IFN-γ and IL-12. Mycobacterial immunity requires IFN-γ and IL-12 between Th1 cells and macrophages. IL-12, produced by infected macrophages, differentiates naïve T cells to Th1 cells and activates them to produce IFN-γ, which binds to its cognate receptor (IFNγR) expressed on macrophages, leading to signal transducer and activator of transcription 1 (STAT1) phosphorylation, dimerization, nuclear translocation, and the transcription of several antimycobacterial genes such as natural resistance-associated macrophage protein 1 (NRAMP1). Macrophages also produce TNF-α in an autocrine manner, which activates themselves, thereby contributing to the sterilization of TB. This control mechanism of mycobacterial infection is termed the IFN-γ/IL-12 axis