PREFACE

The Global Alliance for TB Drug Development (http://www.tballiance.org) is a not-for-profit venture with the mission of accelerating discovery and/or development of cost-effective new tuberculosis (TB) drugs that will shorten the duration of TB treatment or otherwise simplify its completion, provide for more effective treatment for drug-resistant tuberculosis, and/or improve the treatment of latent TB infection. The Global Alliance is issuing this *Scientific Blueprint for TB Drug Development* to provide a detailed, well-referenced document to guide scientists and investigators in academia, industry, and the public sector in all aspects of TB drug discovery and development.

This *Scientific Blueprint for TB Drug Development* presents the following information in detail: (1) the status of the tuberculosis epidemic, (2) the need for new hemotherapeutic agents, (3) an analysis of the current TB drug discovery and development environment, (4) approaches for bridging gaps in the research and development (R&D) process that prevent the timely development of new anti-TB drugs, (5) guidelines for the development process that might increase the chances of obtaining regulatory approval for an effective new treatment.

The Global Alliance for TB Drug Development is dedicated to closing the R&D gaps. However, advances cannot be made without investment by national and international health organizations, private sector pharmaceutical and biotechnology firms, foundations, and others. By combining resources into R&D efforts to discover and develop a broad portfolio of promising candidates, the Global Alliance and its sponsors can make a vitally important contribution to improved control and the eventual elimination of tuberculosis from every country of the world.

1.0 INTRODUCTION

After several decades of neglect, TB is receiving the increased attention that this global public health problem deserves. Governments, nongovernmental organizations (NGOs), and philanthropic organizations are beginning to invest the major sums of money required to control and eventually eliminate this scourge. Although most of these new resources are being appropriately invested in TB control programmes in countries where the TB epidemic is most severe, a significant commitment also is being made to basic research and the development of new diagnostic, treatment and prevention tools, including new TB drugs.

Until now, progress in TB drug development has been impeded by two major factors: (1) the belief that there was little need for new agents, and (2) the high cost of development coupled with the perception that the potential global market was insufficient to guarantee return on investment.

To address these problems, a number of interested parties, with initial support from the Rockefeller Foundation, have created the Global Alliance for TB Drug Development, a not-for-profit venture that will accelerate the discovery and development of new drugs to fight TB. It is one of a new breed of public-private partnerships that pursue a social mission by employing the best practices of the private sector and drawing upon resources from the public and private realms.

The vision of the Global Alliance is the provision of new medicines with equitable access for the improved treatment of TB. Its mission is to accelerate discovery and/or development of cost-effective new TB drugs that will shorten the duration of TB treatment or otherwise simplify its completion, provide for more effective treatment for drug-resistant tuberculosis, and/or improve the treatment of latent TB infection.

The Global Alliance will function as a lean, virtual R&D organization that outsources R&D projects to public or private partners. Based on a survey of TB drug develop-

ment activities in the public and private sectors, it will selectively intervene when its actions will help move a drug candidate towards registration and use in therapy. The Global Alliance therefore will build a portfolio of projects with varying levels of funding, management and ownership.

The Global Alliance is issuing this *Scientific Blueprint for TB Drug Development* to provide a detailed, well-referenced document to guide scientists and investigators in academia, industry, and the public sector in all aspects of TB drug discovery and development. The *Scientific Blueprint* contains an analysis of the current TB drug discovery and development environment and identifies research gaps to inform the work of the Global Alliance. It also includes an 'Executive Summary' which serves as a stand-alone synopsis of the document.

The *Scientific Blueprint for TB Drug Development* presents the following information in detail:

- The status of the tuberculosis epidemic
- The need for new chemotherapeutic agents
- An analysis of the current TB drug discovery and development environment
- Approaches for bridging gaps in the R&D process that prevent the timely development of new anti-TB drugs
- Guidelines for the development process that might increase the chances of obtaining regulatory approval for an effective new treatment.

The Global Alliance for TB Drug Development is dedicated to closing the R&D gaps. However, advances cannot be made without investment by national and international health organizations, private sector pharmaceutical and biotechnology firms, foundations, and others. By combining resources into R&D efforts to discover and develop a broad portfolio of promising candidates, the Global Alliance and its sponsors can make a vitally important contribution to improved control and the eventual elimination of tuberculosis from every country of the world.

2.0 EXECUTIVE SUMMARY

2.1 The need for new TB drug treatments

Tuberculosis is one of the most common infectious diseases known to man. About 32% of the world's population—or 1.86 billion people—are infected with TB. Every year, approximately 8 million of these infected people develop active TB, and almost 2 million of these will die from the disease.[1] In India alone, one person dies of TB every minute.

TB case notifications are soaring in the newly independent states of the former Soviet Union,[2] and human immunodeficiency virus (HIV)-associated TB is out of control in the sub-Saharan African countries hardest hit by acquired immunodeficiency syndrome (AIDS).[3] Moreover, there has been a recent and disturbing increase in the number of TB cases that are caused by organisms that are resistant to the two most important drugs, isoniazid (INH) and rifampicin (RMP). A survey in 72 countries suggested that the multidrug-resistant TB (MDR-TB) problem is more widespread than previously thought and likely is worsening.[4] MDR-TB appears to be especially serious in the Russian Federation, where it has spread in prisons and throughout the general population.[5] If not prevented and controlled, MDR-TB likely will become more widespread in other areas of the world, including developed countries in Western Europe and in the United States, Canada, and Australia.

Widespread use of the bacille Calmette-Guérin (BCG) vaccine, which is the only available TB vaccine, has had limited impact on the global burden of TB. Although BCG vaccination does prevent the development of severe and fatal forms of TB in young children, it has not been effective in reducing the greater numbers of infectious pulmonary cases in adults.[6] Recently there has been increased attention given to the development of a new effective TB vaccine, which is thought to be essential to the eventual elimination of TB.[7] However, this effort might take 25 years or more, and in the interval 50 million lives will be lost to TB.

2.2 Current status of TB control: DOTS

In response to the global TB epidemic, the World Health Organization (WHO) has developed an effective control strategy largely based on the pioneering work of the British Medical Research Council (BMRC) and the International Union Against Tuberculosis and Lung Disease (IUATLD). This strategy is known as DOTS (directly observed treatment, short course).[8]

The essential elements of DOTS are as follows:

- Strong government commitment to TB control
- Diagnosis by smear microscopy (or by culture where resources permit)
- Standardized short course chemotherapy with directly observed treatment for at least the first 2 months
- Secure supply of safe, high-quality drugs
- Individual reporting of treatment outcome and monitoring of programme performance.

Although DOTS is highly effective—82% of patients managed under DOTS in 1997 in the 22 countries with the highest TB burden were successfully treated[9]—its implementation has been slow and overall coverage is low, estimated at only 28% worldwide in 1998. Moreover, DOTS is cumbersome and labour intensive, particularly because currently available anti-TB drugs require a minimum treatment duration of 6 months.

Although great strides have been made in the treatment of TB during the past half century, the most significant progress occurred more than 30 years ago. The current treatment is a 6-month, four-drug combination of INH, RMP, pyrazinamide (PZA), and ethambutol (EMB). All four drugs are given during the initial 2-month 'intensive phase', and then INH and RMP are continued during the 4-month 'continuation phase'. When followed as recommended, this regimen is highly effective, and rates of severe adverse reaction are low. However, many patients experience unpleasant side-effects, and adherence with the relatively long course of treatment is often poor. Such non-adherence commonly leads to treatment failure and the development of drug resistance. The second-line drugs used for MDR-TB are more expensive, less effective, and more toxic than the four-drug standard treatment.

2.3 Objectives for TB drug development

A new TB treatment should offer at least one of the following three improvements over the existing regimens:

- Shorten the total duration of effective treatment and/or significantly reduce the total number of doses needed to be taken under DOTS supervision
- Improve the treatment of MDR-TB, which cannot be treated with INH and RMP
- Provide a more effective treatment of latent TB infection (LTBI).

Ideally, a new, highly effective drug will achieve all three goals.

Although few truly novel compounds to treat TB have been introduced into clinical practice in the past 30 years, some promising work has been done on the following classes of drugs:

- Long-acting rifamycins (e.g., rifapentine, rifabutin, rifalazil)[10–12]
- Fluoroquinolone (FQ) compounds (e.g. levofloxacin, moxifloxacin, gatifloxacin)[13–15]

- Oxazolidinone compounds[16]
- Nitroimidazopyrans.[17]

These drug classes might provide the best means for rapidly improving TB treatment.

Genomics—the systematic identification of all of the genes in a cell through deoxyribonucleic acid (DNA) sequencing and bioinformatic analysis—also offers great potential in terms of drug target discovery and development of new antibacterial agents, and the recently sequenced genome of *Mycobacterium tuberculosis* should provide a number of new targets for novel drugs.[18]

2.4 Overcoming the barriers to TB drug development

Several gaps in the R&D pipeline are impeding the discovery, development and introduction of new drugs to treat TB (Fig. 1):

- **Basic research:** Targets and compounds identified through recent basic research are not being fully exploited
- **Discovery:** Private companies are not willing to dedicate the screening resources or medicinal chemists to optimizing new compounds with TB activity (lead compounds)

- **Preclinical development:** Private companies do not have an interest in preclinical TB studies, and the public sector has limited resources for the coordinated development of preclinical studies
- **Process development/chemistry:** Activities to develop appropriate manufacturing processes are inhibited by the lack of compounds available for scale-up, as well as the unwillingness of pharmaceutical companies to dedicate process chemistry resources to TB chemotherapeutics
- **Clinical trials:** Although the infrastructure for Phase I and II clinical trials is well established, Phase III trials require additional coordination, regulatory support, and funding. However, these limitations are irrelevant without promising novel compounds emerging from preclinical studies
- **Technology transfer:** Little commercialization activity is taking place because of the lack of novel compounds in development, pharmaceutical companies' pessimistic view of the TB market, and concerns about toxicity associated with long-term use.

As the previous list illustrates, the R&D gaps are due to two facts: (1) very few new drugs are in the pipeline, and (2) drug companies have not been interested in TB because the disease appears not to be a major problem in industrialized nations. However, TB does pose a major
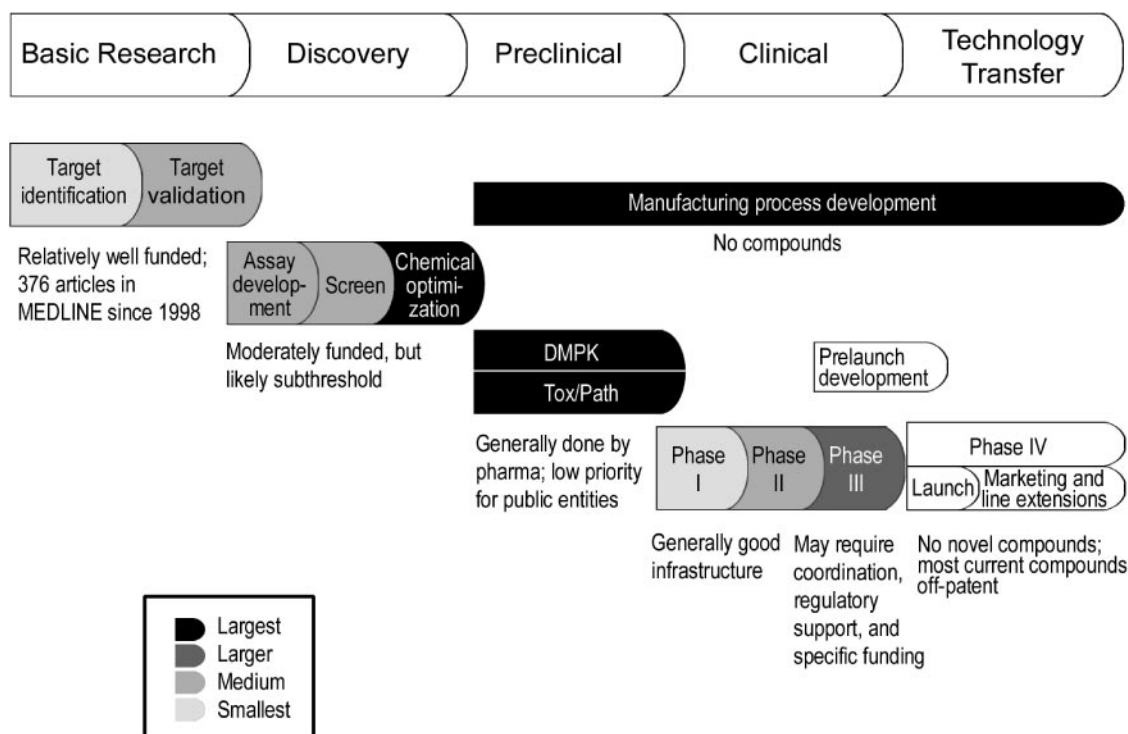

Fig. 1 Gaps in the R&D pipeline.

threat to all nations. The time has come to ensure that new anti-TB drugs make it through the R&D pipeline.

In the face of the significant obstacles to new TB drug development, the Global Alliance for TB Drug Development is working with several partners, including private pharmaceutical companies, to close the gaps in TB drug discovery and development. The Global Alliance's efforts will encourage creative engagement of the public and private sectors in improving the drug development process at every stage in the R&D pipeline, giving priority to the major bottlenecks that occur relatively early in the process (i.e. late discovery and preclinical research):

- **Basic research**
  - Encourage researchers to focus on translational research
  - Encourage researchers to move beyond target identification and validation to assay development
  - Provide funding for target-directed screening activities
- **Discovery**
  - Provide funding for medicinal chemists, particularly those in developing countries, to pursue TB lead optimization
- **Preclinical development**
  - Coordinate and support integrated toxicological and pharmacological resources during lead development
  - Encourage early evaluation of lead compounds in animal models of TB
- **Chemical/process development**
  - Increase the number of compounds available for

scale-up
  - Leverage the process chemistry resources available in developing countries
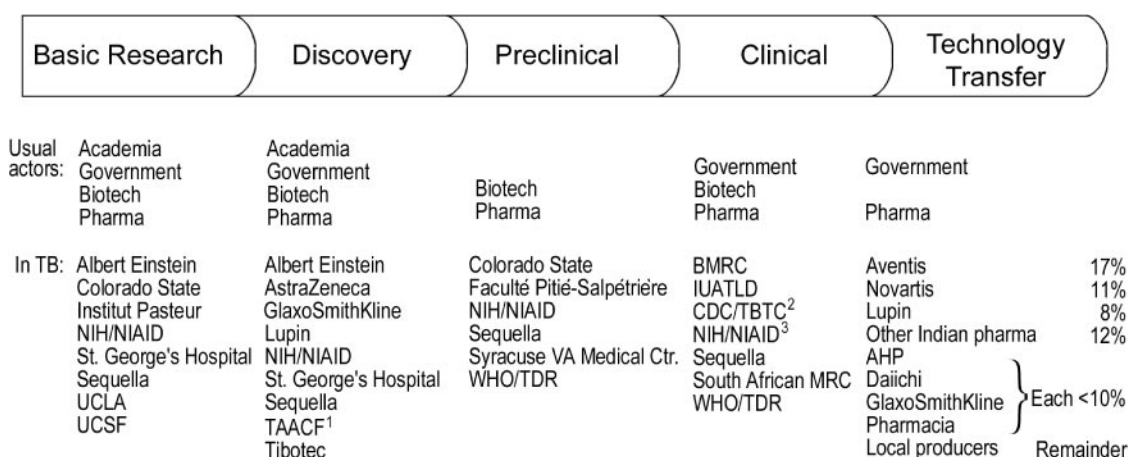- **Clinical trials**
  - Encourage efforts to move promising novel compounds out of preclinical activities and into clinical trials
  - Support the development of a network of sites for conducting cost-effective trials in high-burden countries
  - Identify surrogate markers to streamline trials
- **Technology transfer**
  - Encourage cooperative partnerships among companies with the ability to commercialize new treatments, particularly firms with existing franchises in infectious or tropical diseases
  - Leverage the expertise of public organizations that are positioned to commercialize drugs that benefit the public.

As stated previously, closing the gaps along the tuberculosis R&D pipeline requires the involvement of the public and private sectors. Figure 2 lists some of the organizations currently working in TB drug discovery and development. It should be noted that these organizations do not represent all those conducting tuberculosis R&D. The Global Alliance developed this preliminary list based on an informal survey of some 50 leading scientists, business people, and programme administrators in the TB field.

The probability that a single candidate will progress from discovery through registration is less than 0.5%.

	Basic Research	Discovery	Preclinical	Clinical	Technology Transfer
Usual actors:	Academia Government Biotech Pharma	Academia Government Biotech Pharma	Biotech Pharma	Government Biotech Pharma	Government Pharma
In TB:	Albert Einstein Colorado State Institut Pasteur NIH/NIAID St. George's Hospital Sequella UCLA UCSF	Albert Einstein AstraZeneca GlaxoSmithKline Lupin NIH/NIAID St. George's Hospital Sequella TAACF[1] Tibotec	Colorado State Faculté Pitié-Salpétrière NIH/NIAID Sequella Syracuse VA Medical Ctr. WHO/TDR	BMRC IUATLD CDC/TBTC[2] NIH/NIAID[3] Sequella South African MRC WHO/TDR	Aventis 17% Novartis 11% Lupin 8% Other Indian pharma 12% AHP Daiichi GlaxoSmithKline } Each <10% Pharmacia Local producers Remainder

(1) Tuberculosis Antimicrobial Acquisition and Coordination Facility, funded by NIH/NIAID.
(2) Tuberculosis Trials Consortium.
(3) Includes TB Research Unit, Vaccine, and Treatment Evaluation Unit; HIV Trial Site Network.

Fig. 2 Sample of organizations working in TB drug R&D.

Therefore, the Global Alliance will distribute its support across multiple targets and mechanisms of action, among multiple partner organizations, and along multiple phases of the R&D pipeline. The focus will be on developing a broad portfolio of promising candidates with a special emphasis on developing fast-track compounds that might exhibit success early in the development phase (e.g. quinolones, oxazolidinones). The Global Alliance calls on funding agencies and research organizations to devote the resources needed to support these efforts.

2.5 A successful drug discovery and development process

As efforts to develop a new treatment for tuberculosis improve, researchers and their sponsoring organizations should consider following the process outlined in this section as they proceed through drug discovery and development. The Global Alliance believes that this process will help ensure that new compounds to treat TB will move successfully through development, receive rapid regulatory approval, and be transferred into clinical use.

2.5.1 Target selection

In general, drug discovery programmes are aimed at proteins whose function is known to be essential to the bacterial cell. Several important criteria are used to evaluate the suitability of a candidate enzyme or protein as a drug target:

- The level of existing validation of the target
- The deduced tractability of the target
- Availability of three-dimensional structure data
- An approachable assay system that can be readily adapted to high-throughput screening technology
- Lack of mammalian homologues.

Because one-third of the global human population is asymptomatically infected with TB (i.e. has latent TB infection) and at continued risk for activation of the disease, much basic research has focused on understanding the physiology and metabolism of the latent bacilli within such patients. Candidate proteins and processes have emerged that might be critical for the continued persistence of bacteria within such patients.[19] These processes might offer a viable treatment target for patients with LTBI, but such targets have several problems. For example, these targets might not be essential for normal growth of the bacilli, and thus conventional microbiological laboratory procedures for determining resistance might not be applicable. However, these targets are approachable through conventional assay development, inhibitor design, lead optimization, and preclinical

development processes, and they are an important component of future TB drug development considerations.

2.5.2 Identification of lead compounds

In most cases, lead compounds are identified through successfully implemented high-throughput assays and surveys of chemical diversity for compounds that inhibit the target selected. The process of lead compound identification has been greatly enhanced by the advent of combinatorial chemical approaches to generating compound diversity.[20] This technology has allowed the creation of literally millions of discrete substances that can be individually assessed for their potential to inhibit the target. Each of the resulting inhibitors then represents a starting point (i.e. lead compound) whose structure is further manipulated to improve binding and other important characteristics.

Lead compounds can also be identified based on known inhibitors, chemical intuition, or even known drugs. If the target enzyme has a solved three-dimensional structure, lead compounds also can be identified in silico through the application of molecular docking algorithms. All of these processes together can produce a series of lead compounds that might be suitable for further medicinal chemical manipulation to produce candidates for preclinical evaluation.

2.5.3 Optimization of lead compounds

Lead compounds are optimized through synthesis of related substances while maintaining the essential features of the original compound that conferred the inhibitory property. Such processes are facilitated by the knowledge of a crystal structure of the target, especially if a cocrystal of the lead compound can be obtained. Both computer simulation and trial-and-error testing are used when attempting to maximize the 'fit' of the compound into the active site of the protein. Selection of a lead compound and analogue generation generally occurs in parallel with an initial evaluation of a compound's drug-likeness and an investigation of preliminary characteristics known to be associated with successful development programmes. In general, these processes should happen simultaneously—that is, an assessment of binding affinity for a potential analogue should be coupled with an evaluation of the likelihood that such an analogue represents a viable development candidate. Such evaluations typically involve the following:

- Assessment of toxicity (on a eukaryotic cell-line initially)
- In vitro determination of the minimum inhibitory concentration (MIC) of the lead compound against *M. tuberculosis*

- In silico prediction of the likelihood that the compound will enter the bloodstream following oral administration
- Evaluation of bioavailability and efficacy in animal models
- Initial measurement of the compound's serum stability using hepatic microsomal stability assays.

All of these data must be integrated with analogue generation to select a lead series that has the greatest hope of advancement to preclinical status.

2.5.4 Late discovery

'Assessing the robustness' of a short list of compounds is essential in defining and balancing a set of preferred characteristics and in eliminating compounds that might not successfully complete development. The process of validating lead compounds according to predefined criteria generally incorporates standard tests (that have been developed to assess key characteristics of any compound) and specific tests (that incorporate disease-specific characteristics desired in a new drug candidate):

- **Formulation feasibility:** A pharmaceutical chemist should be able to formulate an orally bioavailable, stable finished drug product that meets all of the characteristics required of a new anti-TB compound
- **Pharmacokinetics; absorption, distribution, metabolism and excretion (ADME); and pharmacodynamics:** The key parameters to assess are (1) the relationship between the blood and tissue levels of the compound in comparison to its MIC/MBC (minimum bactericidal concentration) against TB, and (2) the plasma or serumcidal activity of a compound against ex vivo *M. tuberculosis*
- **Toxicology:** Commonly performed studies include mutagenicity studies, acute single dose toxicity studies, subacute (5- to 7-day) toxicity studies, and preliminary dose-ranging studies
- **Safety (secondary) pharmacology:** These tests discover whether a compound has pharmacological activity against other human receptors that control biologic functions
- **Intellectual property (patent) situation:** The strongest intellectual property protection an organization can seek is for a new previously undescribed chemical class.

2.5.5 Preclinical development

Researchers must use animal models to assess in vivo the antimicrobial activity of a lead compound in comparison with that of existing drugs. In addition, the models test the compound's antagonistic, additive, or synergistic effects when given in combination with other drugs and its ability to sterilize the lesions of the experimentally infected animal. Because of its ease of handling in terms of size, supply, maintenance, robustness, and reproducibility, the mouse is the model of choice for TB.[21,22] When used with care, the mouse model is able to reproduce bacteriologic conditions close to those present in the natural human disease and provide information on drug activity that can be extrapolated to human beings.

Animal studies should assess bacterial burden, mortality, and organomegaly in lung tissue at baseline; during therapy; at the end of therapy; and post-therapy to assess relapse, postantibiotic effect, and development of resistance. They should include combination drug evaluations to better identify the place for a new TB drug within the established therapeutic regimens.

Finally, properly planned toxicology studies must be conducted. Some of these studies might have been conducted during the late discovery phase; however, it is essential that researchers conduct preclinical toxicology studies in compliance with good laboratory practice—an absolute requirement for regulatory purposes. These studies are highly controlled and documented safety tests to demonstrate possible toxic effects of the compound in order to define a window of safety for subsequent human clinical trials.

Early discussions and interactions with representatives of regulatory agencies are encouraged and can provide critical guidance on the types and design of studies (both preclinical and clinical) likely to facilitate TB drug development.

2.5.6 Process development/chemistry

Before clinical trials begin, the chemical and pharmaceutical development team continues its task of scaling up the previously determined method of drug substance production, dosage form development, and development of analytical methods to ensure that good manufacturing practices can be maintained for each batch produced.

A series of steps related to process development and chemistry also must be taken. The chemical and pharmaceutical development team produces a description of the lab scale route of synthesis (i.e. extraction or other process that yields the candidate compound). The team also performs a feasibility assessment to ensure that the compound, based on the proposed method of production, can be produced in large quantities. In addition, the team produces an early estimate of the candidate compound's stability, a description of its physical-chemical properties, and an assessment of its chiral or diastereomeric purity.

2.5.7 Clinical trials

All clinical trials must conform with internationally accepted standards of good clinical practice. Researchers should ensure that institutional review boards and/or independent ethics committees are properly established in the countries where the research is to be conducted and that these committees have the resources and independence to review the proposed studies. Whenever possible, the control and comparison treatments should be masked and placebos used to avoid the introduction of bias for or against the new treatment. The assessment of adverse events always must be included. Documenting, monitoring, and auditing the study process are essential to ensure the quality of the data. Exceptions to the protocol and protocol errors must be documented. Adequate safety information will need to be included in the submission for regulatory approval. The safety database should include representation from racial and ethnic groups likely to receive the product, patients of both genders, and patients infected with HIV. Researchers should generate sufficient pharmacokinetic information to assess the likelihood of interactions with the most common concomitant therapies, including both TB and non-TB drugs.

Phase I and II trials

Phase I trials are conducted in healthy normal volunteers (HNV) of either gender and are designed to give the investigator an idea of the pharmacokinetic profile and limited safety data (clinical and laboratory) on a new drug. In addition to Phase I HNV trials, researchers might also incorporate the pharmacokinetic and safety instruments into a larger Phase II study that enrols patients with active TB and uses additional data instruments, such as efficacy and multiple treatment groups.

A critical function of Phase I/II trials is determining the optimal drug dose for the Phase III trials. One well-documented method to rapidly demonstrate drug activity in man and to assist in selection of the optimal drug dosage is the early bactericidal activity (EBA) study.[23] For the EBA study, newly diagnosed TB patients are treated for 2 to 5 days with various dosages of a new drug, while carefully measuring the colony-forming units (CFU) in expectorated sputum.

Phase III trials

Phase III trials are usually large scale, randomized clinical trials designed to show improved or equivalent efficacy of a new treatment compared to the standard treatment among diseased patients. For TB, up to 1000 patients are commonly enrolled in a two-arm study, treated, and then followed for TB relapse for up to 2 years, the commonly accepted primary end point for demonstrating efficacy.[24]

The Phase III trial design should outline the parameters that will be used to define primary and secondary end points, including the sample sizes, confidence intervals, and statistical methods that will be used to assess the data. It is imperative that microbiologic evaluations take place at the appropriate times during the Phase III clinical trials in order to assess the true activity of the investigational agent.

To ensure that a sufficient study population can be obtained for the Phase III trials, researchers might need to conduct trials in countries with high TB incidence rates. One desired characteristic for establishing a clinical trial site in a particular country is that the national tuberculosis programme should be strong and steadily expanding to serve the entire country (if it does not already do so). Only a programme of this type can provide essential information, such as the annual incidence of cases by type (e.g. site of disease, smear status, drug resistance) and the prevalence of complicating comorbidity. These data allow for accurate estimates of patient enrolment in the study and whether a particular treatment is appropriate for that site.

The reporting system and collection of results must be carefully designed to avoid errors. A reference laboratory also is required for most trials, but the extent to which it duplicates the procedures carried out in the local laboratory depends on the local laboratory's standards and its capacity to do the necessary work.

Finally, validated surrogate markers of relapse could provide evidence on the sterilising activity of a drug/regimen with great savings in development time and cost. The most useful method for studying a drug's sterilising activity is to determine the proportion of patients who have a negative culture of a single sputum specimen at 2 months (8 weeks) after the start of treatment with an experimental regimen compared with the proportion on a standard regimen.[25] Newer molecular methods might provide better surrogate markers of response but require further study and validation.[26]

2.5.8 Regulatory approval

Given the lengthy development process, any delay in receiving regulatory approval will be seen by industry as an additional tax on an already limited profit potential. Regulatory uniformity among national agencies would help remove some of the current disincentives to TB drug development. The *Scientific Blueprint* provides information, particularly in the area of clinical studies, that can be used in developing standardized international guidelines for regulatory approval of new TB drugs. It is hoped that such guidelines will enhance the efficiency in registering new anti-TB agents while continuing to follow current national requirements that are designed to protect individuals and public health.

2.5.9 Technology transfer

Product development efforts, including patenting, describing biological activity, assessing toxicity, developing a safety profile in humans, and demonstrating clinical efficacy at the proposed dosage and mode of administration, are well-established steps of the preclinical development and clinical testing processes. Performing these studies under codes of good manufacturing, laboratory, and clinical practice enhances the technology transfer effort. The commercialization strategy must be developed before clinical testing to ensure that the needs of the target market are clearly understood and taken into consideration in developing the drug product.

Two types of postmarketing (Phase IV) studies are required: (1) evaluations under programme conditions of new treatment regimens in comparison to locally mandated regimens, and (2) surveillance for less common adverse effects related to the new drug, including the development of drug resistance. Patient acceptance of the new drug must be objectively assessed. Economic and financial benefits of using the new drug also should be assessed.

To generalize the use of a new drug internationally, intergovernmental organizations, NGOs, and drug manufacturers have certain responsibilities:

- WHO establishes technical norms and informs drug manufacturers of them via the International Federation of Pharmaceutical Manufacturers Associations (IFPMA) and/or the International Generic Pharmaceutical Alliance (IGPA)
- IFPMA and IGPA disseminate technical and other requirements to their constituent members through the appropriate channels
- Public health agencies, NGOs and professional societies issue technical guidelines on the use of new drugs in TB treatment
- WHO should consider including any new anti-TB drug on its Essential Drugs List (EDL), and international suppliers such as UNICEF and the International Drug Association should consider placing the new drug in their catalogues of available therapeutics
- Development institutions such as the World Bank, regional development banks, and other international aid agencies and foundations should accommodate the purchase of the new drug in loan or aid agreements
- Countries establish national drug policies and regulations to suitably control the new drug. Policy and regulation development requires full coordination among the national tuberculosis programme, the national drug regulatory authority, and the national procurement office.

New TB drugs will become widely accessible and properly used only if all of these systems are sufficiently integrated and supported by strong national TB programmes with appropriate training at all levels of the health system.

3.0 STATUS OF TUBERCULOSIS AND ITS TREATMENT

Before one can begin to consider how best to develop new drugs to treat tuberculosis, it is important to understand the history of the disease and current chemotherapy regimens. This section presents an overview of the current TB epidemic and how it is being controlled, a short history of the development of TB drugs and treatment regimens, and the modes of action and activity of the current anti-TB drugs. Also considered are the objectives for new TB therapies and the status of current TB drug development efforts.

3.1 Current TB epidemic

Over the centuries, tuberculosis has accounted for more human misery, suffering, loss of earnings, and failure of economic and social development than any other disease. TB is one of the most common infectious diseases known to man. About 32% of the world's population—or 1.86 billion people—are infected with *M. tuberculosis*. Every year, approximately 8 million of these infected people develop active TB, and almost 2 million of these will die from the disease.[1] TB is the world's longest running catastrophe, killing more than 200 people every hour and more than 5000 every day. In India alone, one person dies of TB every minute. Each of the 3.5 million new infectious cases that occur each year might transmit TB to more than 20 other people.[27]

As the global population grows, so does the burden of TB. TB case notifications are soaring in the newly independent states of the former Soviet Union.[2] HIV is fueling the TB epidemic in many countries in sub-Saharan Africa.[3] Five-fold increases in TB incidence have been seen in Kenya in spite of a well-functioning control programme and good cure rates. In Zambia and Zimbabwe, more than 70% of TB patients are also infected with HIV, and case notifications are rising. HIV and TB together account for nearly half of the adult mortality in the worst affected countries. In Malawi, the mortality rate during TB treatment is now over 20%.

With the global spread of HIV, especially in Asia, similar increases in TB incidence rates and mortality are to be feared in countries like India, which is home to 4 million people with TB and now also to 3.5 million HIV-infected individuals, the largest number of any single country.

Moreover, there has been a recent and disturbing increase in the number of TB cases that are caused by organisms that are resistant to the two most important drugs, INH and RMP. A 1994–97 survey found that rates of MDR-TB were alarmingly high in several areas of the world.[28] A more recent survey in 72 countries suggested that the MDR-TB problem is more widespread than previously thought and likely is worsening.[4] For example, 5.8% of TB cases in Iran are MDR, as are 10.8% in China and 14.1% in Estonia. MDR-TB appears to be especially serious in the Russian Federation, where it has spread in prisons and throughout the general population.[5] Although the estimated rate of MDR-TB in newly diagnosed patients worldwide has been estimated to be under 2%, new data suggest that the rate is increasing.[4] If not prevented and controlled, MDR-TB is likely to become more widespread in other areas of the world, including developed countries in Western Europe and in the United States, Canada, and Australia.

Widespread use of bacille Calmette-Guérin vaccine, which is the only available TB vaccine, has had limited impact on the global burden of TB. Although BCG vaccination does prevent the development of severe and fatal forms of TB in young children, it has not been effective in reducing the greater numbers of infectious pulmonary cases in adults.[6] Recently there has been increased attention given to the development of a new effective TB vaccine, which is thought to be essential to the eventual elimination of TB.[7] However, this effort might take 25 years or more, and in the interval 50 million lives will be lost to TB.

3.2 Current status of TB control: DOTS

In response to the global TB epidemic, the WHO has developed an effective control strategy largely based on the pioneering work of the BMRC and the IUATLD. This strategy is known as DOTS (directly observed treatment, short course).[8] The essential elements of DOTS are as follows:

- Strong government commitment to TB control
- Diagnosis by smear microscopy (or by culture where resources permit)
- Standardized short course chemotherapy with directly observed treatment for at least the first 2 months
- Secure supply of safe, high-quality drugs
- Individual reporting of treatment outcome and monitoring of programme performance.

WHO's strategy has been adopted by high-burden countries at a remarkable rate. Although it was used by only 10 countries in 1990, DOTS had been adopted by 119 countries by end 1998,[29] including all 22 of the high-burden countries that account for 80% of the world's TB burden. Some 21% of all TB cases are now treated under DOTS programmes.

Although DOTS is highly effective—82% of patients managed under DOTS in 1997 in the 22 countries with the

highest TB burden were successfully treated[9]—its implementation has been slow and overall coverage is low, estimated at only 28% worldwide in 1998. Moreover, DOTS is cumbersome and labour intensive, particularly because currently available anti-TB drugs require a minimum treatment duration of 6 months (see Section 3.3.2). Even if WHO achieves its treatment targets under DOTS by the year 2010, it will have prevented only 23% (48 million) of the TB cases predicted between 1998 and 2020.[30]

3.3 History of modern chemotherapy

Since the initial studies of streptomycin (STR) in the late 1940s, the public sector, with willing collaboration from the private sector, has played a large role in the development of TB drugs. Organizations such as the BMRC,[31] the US Public Health Service (USPHS), the US Armed Forces Veterans Administration Cooperative Trials, and the IUATLD conducted large scale, randomized trials to define the optimal use of the drugs in TB treatment regimens.[32]

3.3.1 Initial development of TB drugs

Following the discovery of STR in 1944, researchers demonstrated its efficacy against TB in mice and guinea pigs. The first report of STR's clinical efficacy came in 1945 from a small, uncontrolled series of patients with progressive TB at the Mayo Clinic in the United States.[33] In 1947, the BMRC and the USPHS began the first randomized, controlled trials of STR, with the BMRC reporting its results 1 year later.[34] These studies demonstrated STR's remarkable ability to reduce mortality and improve clinical status. However, monotherapy with STR led to drug-resistant TB in a high proportion of patients, whose ultimate fate was little better than that of patients who did not receive STR.[35] The ability of a second drug, para-aminosalicylic acid (PAS), to prevent the development of resistance to STR was demonstrated in several studies by the BMRC beginning in 1948. The main aim of subsequent work was to develop regimens that prevented the development of drug-resistant TB.

The next major advance in TB treatment occurred in 1952 with the initial report of a BMRC study of INH.[36] During the next several years, a number of studies were undertaken to evaluate combinations of the three available drugs. An IUATLD study showed that a regimen starting with a three-drug combination of STR, INH, and PAS followed by INH plus PAS was highly effective in centres with a high standard of patient care.[37] This regimen was adopted as standard treatment in many technologically advanced countries but was too expensive for developing countries.

With the scientific basis for TB chemotherapy firmly established, attention turned to the next drugs available for clinical study: PZA, ethionamide (ETH), and cycloserine. Initial evaluation of PZA suggested that the drug was too toxic for use as a first-line agent. This was also the case with the other two agents. However, the basis was established for treating patients with drug-resistant TB using these second-line drugs.

While continuing to conduct treatment efficacy studies, the USPHS also embarked on pioneering studies using INH to prevent tuberculosis in persons with latent TB infection. Between 1955 and 1959, the USPHS studied nearly 65,000 persons, including children with primary TB, persons in contact with infectious TB patients, TB patients in mental institutions, and Alaskan villagers. These placebo-controlled trials demonstrated the efficacy of INH for treatment of LTBI.[38]

Beginning in 1958 and continuing into the 1960s, the BMRC conducted a series of studies in Africa suggesting that PAS could be replaced with thiacetazone. Because of its low cost, thiacetazone became an established TB drug for use in low-income countries throughout the world. In industrialized countries, PAS was replaced by EMB, which was introduced in 1962 and subsequently studied by USPHS. Ethambutol also replaced thiacetazone in many low-income countries during the AIDS epidemic, when HIV-infected patients were found to experience a high rate of serious and sometimes fatal skin reactions to thiacetazone.[39]

3.3.2 Improving treatment regimens

Several studies, conducted largely by the BMRC, indicated that the optimal duration of TB therapy with any of the drug combinations described above was 18 months to 2 years, with relapse rates following treatment of less than 5%. Throughout the 1950s, patients were confined to hospitals and sanatoria for daily treatment. A study published in 1959, coordinated by the BMRC in Chennai (formerly Madras), established that patients treated at home did as well as those treated in hospital without increasing TB transmission among their household contacts.[40] With this demonstration of the efficacy of ambulatory treatment, the central issue for further development of TB drugs was to ensure that patients continued to take their medication throughout the lengthy treatment period. A 1970 BMRC study in East Africa showed that thiacetazone-containing regimens were much less effective under routine conditions than they had been in clinical trials, largely because patients failed to complete the entire treatment regimen.[41] Fully supervised treatment—later renamed directly observed therapy—was clearly necessary to maintain the efficacy of the lengthy regimens.

The initial attempt to facilitate adherence to treatment was the development of intermittent regimens that required fewer supervised drug doses. A large number of studies, mainly initiated by the BMRC, showed that the efficacy of the regimen could be maintained if drugs were given two or three times per week. Currently, the most widely used intermittent regimens are either fully intermittent, with doses administered three times per week, or twice weekly following an initial period of daily treatment.

The era of short course TB therapy began with the introduction of RMP, which became available for clinical trials in 1966. An East African-BMRC study found that the addition of RMP or PZA to a 6-month STR-INH regimen substantially reduced relapse rates.[42] A USPHS study found a combination of RMP at a dosage of 10 mg/kg (600 mg for most adults) and INH administered daily for 9 months to be superior to existing regimens.[43]

Henceforth, the aim in developing new TB regimens was to shorten the period of treatment while keeping the costs of the drugs and the supervision of drug-taking as low as possible. A series of BMRC studies in Hong Kong, Singapore, Africa, and India found that RMP and PZA acted synergistically in an initial phase but that only RMP was effective in a continuation phase. A standard regimen then was developed in Singapore in which a 2-month initial four-drug phase (INH, RMP, PZA and STR) was followed by 4 months of INH-RMP. For circumstances where the cost of RMP during the whole of chemotherapy was prohibitive, an 'African' alternative was developed, which involved a 2-month initial phase of four drugs followed by a INH-thiacetazone 6-month continuation phase. In most countries, EMB has replaced STR and thiacetazone. These regimens are now the basis of modern chemotherapy.

The BMRC, USPHS, and others have conducted studies of combined formulations of TB drugs that have been promoted to increase adherence and to assist in the prevention of drug resistance. Currently, preparations of INH-RMP, INH-EMB, INH-RMP-PZA, and INH-RMP-PZA-EMB are available. However, one important problem that has been found with some formulations is poor bioavailability of RMP. Thus, these products must meet stringent quality control standards.[44]

3.4 Modes of action and activities of the anti-TB drugs

Current chemotherapy for TB relies largely on mycobacteria-specific drugs that inhibit bacterial metabolism with a heavy emphasis on inhibitors of the cell wall superpolymer. As detailed in Section 3.3.2, the four first-line agents are INH, RMP, PZA and EMB. Streptomycin is widely used as second-line therapy.

3.4.1 Modes of action

Isoniazid is a prodrug that requires oxidative activation by the mycobacterial catalase-peroxidase *katG*. The poorly understood active form of INH subsequently interacts with the enzymatic machinery that synthesises mycolic acids, essential components of the cell wall. *katG* mutations affecting this activation process are found in the vast majority of INH-resistant patient isolates.[45]

Rifampicin interferes directly with the bacterial machinery for transcribing ribonucleic acid (RNA) from DNA. Patient isolates that are resistant to RMP almost invariably have mutations within the beta-subunit of the RNA polymerase gene (*rpoB*).[46]

Pyrazinamide is also a prodrug that requires activation by a hydrolytic pyrazinamidase that converts it to pyrazinoic acid. Pyrazinoic acid has been proposed to have both specific and nonspecific effects due to an intracellular accumulation of the liberated acid. Patient isolates that are resistant to PZA typically show mutation within the gene encoding the pyrazinamidase *pncA*.[47]

Ethambutol interferes with the construction of the arabinogalactan layer of the mycobacterial cell wall. Although definitive proof is lacking, it appears that EMB directly inhibits an enzyme that introduces arabinose into one of the branching structures of arabinogalactan.[48,49]

Streptomycin is one of the family of aminoglycoside antibiotics that acts by inhibiting protein synthesis. Mutations in patient isolates associated with STR resistance include alterations in the gene encoding the 16S rRNA and in the ribosomal protein S12.[50,51]

3.4.2 Bactericidal and sterilizing activities

Bactericidal activity of a TB drug can be defined as its ability to kill a rapidly growing (i.e. log phase) culture of *M. tuberculosis* or the corresponding rapidly growing population of bacilli in the mouse or in human TB lesions. Sterilizing activity of a TB drug can be defined as its ability to kill the slowly growing or slowly metabolizing bacilli that persist after the rapidly growing bacilli have been killed by bactericidal drugs (i.e. persisters). Sterilizing activity also describes the ability of a drug to eliminate latent bacilli held in check by host immunity in persons with LTBI. Although the distinction between rapid and slow growth of bacilli is unlikely to be abrupt, the distinction between bactericidal and sterilizing activity is of great practical importance in TB chemotherapy. The sterilizing activity of a regimen determines for how long it must be given to reduce the residual viable bacterial population to a level that is seldom followed by relapse, whereas bactericidal activity does not determine the length of treatment.[52]

Bactericidal activity is operationally defined as the time and concentration of exposure to a drug from which the bacterium cannot be revived. Bactericidal activity can be measured in vitro as the kill of a log-phase culture. Bactericidal activity occasionally results in overt lysis of the bacterial cells. Of the four first-line TB drugs, only INH has demonstrated cell lysis in vitro, after exposure for 24 h at concentrations above the MIC. Bactericidal activity also might be associated with exceptionally tight binding so that the effect of a drug is irreversible, or with the induction of a cellular process that results in cell incapacitation. Neither PZA nor EMB binds to its target, nor do they apparently trigger an irreversible effect in vitro. In the mouse, bactericidal activity is measured as the kill during the first 7 to 14 days of experimental chemotherapy in a model of acute TB. In human disease, bactericidal activity is best measured in EBA studies. In these studies, patients with newly diagnosed TB are treated for several days with a single drug or drug combination, and quantitative measurements of CFU in sputa are made.[23,53]

Measurement of sterilizing activity requires a model representing persisters or latent bacilli. Although none of the currently available in vitro models has been shown to correlate with in vivo persistence, they nonetheless deserve further evaluation. One model is the Wayne system of a stationary-phase liquid culture adapted to microaerophilic conditions.[54] Alternatively, the in vitro model may consist of phenotypically resistant survivors of such a culture after exposure to bactericidal drugs. Measuring sterilizing activity in vivo to determine overall efficacy of a drug or drug combination is significantly more complex. Both inhibition of transcription and inhibition of cell wall construction might play a substantial role in facilitating irreversible damage of the bacillus by host defensive mechanisms. An overtly bactericidal mechanism of action, as distinct from a bacteriostatic mechanism, contributes to sterilization. Bactericidal activity, however, might result from bacteriostasis when combined with host defensive molecules whose activity may synergize with a weakened bacterium. Sterilizing activity therefore is not necessarily predictable either from mode of action or in vitro analysis of MIC/MBC effects but instead must be analysed within animal models of infection or human clinical trials. Sterilizing activity can be measured in the mouse model of chronic TB in which immune mice are challenged with a virulent strain. In this model, a static bacterial population is killed most rapidly by drugs of high sterilizing activity. In clinical trials, sterilizing activity is best measured by the relapse rate after chemotherapy. Surrogate markers of sterilizing activity, such as the proportion of patients with positive sputum culture at 2 months, might also be useful.

The main TB drugs have well-defined bactericidal properties.[55] Isoniazid in its usual dose of 300 mg daily has high bactericidal activity, mainly because this dose is about 20 times higher than the dose with detectable activity (18 mg). No other TB drug has this large therapeutic margin between the usual and the minimal dose sizes. This large margin probably accounts for the fact that INH's EBA—estimated as 0.575 $\log_{10}$ CFU/ml sputum/day—is the largest of all TB drugs. Nevertheless, the bactericidal activity of INH is virtually complete after 2 days of treatment (possibly a little longer in more chronic disease) and then reverts to low levels, since it seems very slow in killing persisting bacilli.

In treatment regimens containing INH and RMP, the continuing bactericidal activity seems to be due almost entirely to the RMP component, aided in the initial phase by PZA. This hypothesis stems from clinical trials finding that initial resistance to INH had very little effect on either the incidence of failures during chemotherapy or the relapse rate after completion of treatment. However, when RMP and PZA were omitted from the regimen and INH was the principal sterilizing agent, treatment had to be prolonged to 12 to 18 months. Omitting RMP from the continuation phase necessitated increasing the duration of treatment from 6 months to 8 months. Another hypothesis is that INH has a slow sterilizing action when given without RMP or PZA because some of the persisting bacilli move temporarily into a phase of active division when they can be killed. However, such bacilli are likely to be killed much sooner by the rapid action of RMP than by INH. Thus, in the continuation phase of treatment, the predominant bactericidal drug is RMP, and it is doubtful whether INH plays an important role either in killing persisters or in preventing the emergence of resistance.

Ethambutol's usual dose is only slightly above that which is minimally effective, yet it is as bactericidal as RMP, and its EBA increases with dose size. Despite its fairly high bactericidal activity, EMB appears to have no sterilizing activity either in mice or guinea pigs[56] or indeed at conventional dose sizes in clinical trials.[57] Even when the dose size is greatly increased, ethambutyl seems to lack significant sterilizing activity. Thus, in a study exploring intermittent treatment with EMB and INH, a once-weekly regimen of 90 mg/kg EMB (six times the standard daily dose) was highly effective in producing bacteriological quiescent disease at 1 year when accompanied by 15 mg/kg INH.[58] In this study, the well-known weakness of the once-weekly INH component was compensated for by the high efficacy of the high dose of EMB.[59] The high dose of EMB given once weekly, although capable of preventing failure during treatment, was followed by relapse after treatment in 56% of patients. In other words, even at a very high dosage, EMB lacks sterilizing activity.

3.4.3 In vivo metabolism and distinct bacterial populations

The natural history of TB results in several discrete clinical stages during which bacilli are thought to occupy very different environments. Initial entry and growth within a macrophage vacuole is followed by growth slowdown or restriction within a granuloma. This in turn is followed by bacterial multiplication within a liquefied centre of a caseous granuloma in progressive disease. Each of these stages presents the bacterium with a distinct growth milieu, and these probably require specific capabilities for the bacilli to thrive. During infection, these metabolic states also may translate into a selective activity of specific drugs upon discrete subpopulations of bacteria. It is clear that the in vivo metabolism of tubercle bacilli is likely to be substantially different from the in vitro metabolism and that drug susceptibility measurements or screening programmes that rely solely on in vitro analyses of drug efficacy will be inadequate. In addition, oxygen tension and primary carbon source in vitro are likely to be significantly different from those of in vivo bacilli. This explanation is among the hypotheses to account for the sterilizing activity in vivo of certain drugs that have weaker in vitro activity.

There is substantial disagreement over the physiologic state of bacilli that persist following chemotherapy. Latent bacilli are thought to persist anaerobically and without active replication, hence their resistance to chemotherapeutics that rely upon processes required for cell growth. In vitro models of such latent bacilli have revealed sensitivities to certain drugs (e.g. metronidazole and PA-824),[17,60] but an evaluation of metronidazole in an animal model of latency did not demonstrate improved sterilizing activity.[61]

Populations of bacilli with varying drug susceptibilities also might simply reflect pharmacodynamics and tissue distribution of various drugs. In in vivo models, specific physical environments that contain bacteria might be accessible only to drugs with certain physical properties favouring deep tissue penetration, lung bioavailability, penetration into the granuloma, and long serum half-life. For example, bacilli that are located perivascularly might be killed effectively by drugs with poor tissue penetration, while bacilli located in giant cells within a granuloma might not respond to such compounds. Accurately assessing tissue distribution and penetration is critical to developing truly effective TB therapies.

Further study and a deeper understanding of the physiological state of latent bacilli in vivo are absolutely essential if targets with potential for improved sterilizing activity are to be identified.

3.5 Objectives for TB drug development

A new TB treatment should offer at least one of the three following improvements over the existing regimens:

- Shorten the total duration of effective treatment and/or significantly reduce the total number of doses needed to be taken under direct supervision
- Improve the treatment of MDR-TB, which cannot be treated with INH and RMP
- Provide a more effective treatment of LTBI, a strategy that might assist in TB elimination in low-incidence countries.

Ideally, a new, highly effective drug will achieve all three goals. Minimally acceptable product characteristics for new compounds, as well as additional desirable characteristics, are listed in Table 1.

3.5.1 Shortening duration/reducing doses

Of greatest importance are new drugs with improved potency that shorten the overall duration of therapy. The currently acceptable four-drug regimen for most forms of TB requires at least 6 months of treatment.[62] As described in Section 3.3.2, most of the benefit from treatment comes during the first 2 months, when the four drugs are given together in the intensive, or bactericidal, phase. During this time, the bacterial burden is greatly reduced, and patients become noninfectious. The 4- to 6-month continuation, or sterilizing, phase is required to eliminate persisting bacilli and decrease the risk of relapse from 30% or more to less than 5%.[62] A potent sterilizing drug that shortens treatment to 2 months or less with acceptable results (i.e. a success of 95% or greater) will be of great benefit. Such a drug could be added to the current regimen, or it might be substituted for any component of the existing treatment.

Reducing the frequency of drug administration to simplify oversight and improve patient adherence will also be of significant value. Rifamycin derivatives currently under investigation might make possible regimens that are given once weekly throughout most of the treatment period. Depot preparations of rifamycins or of PZA in liposomes or microencapsulates also might be of value. Since multidrug therapy is likely to remain the standard of care, matching treatment intervals of all components of a regimen is also highly desirable.

3.5.2 Improving treatment of drug-resistant TB

The need for drugs to improve the treatment of drug-resistant TB has received a great deal of attention,[63] in large part because of well-publicised outbreaks of MDR-TB. By current definition, MDR strains are resistant to

Table 1   Product profile for new anti-TB drug*

	Minimal characteristic	**Added value characteristic**
Route of administration	Oral	
Likelihood of resistance developing	Spontaneous mutation rate similar to that for existing anti-TB drugs	Spontaneous mutation rate less than that for existing anti-TB drugs
Early bactericidal activity		EBA as a single entity
Activity against latent TB		Active
Activity against resistant TB strains	Active in vivo against all TB isolates including multidrug-resistant strains	
Dosing schedule	Once daily	Once weekly or less
Length of administration	6 months as part of combination chemotherapy	Entire regimen is 4 months or less (combination chemotherapy)
Clinical safety	Safety profile in clinic not significantly worse than existing first-line anti-TB agents in terms of incidence and seriousness of adverse reactions when given in combination for desired length of administration	No significant toxicity
Clinical efficacy	Relapse rates at 6 months post-treatment similar to current regimen (when new agent is used in combination for desired length of administration)	Relapse rates at 6 months shown to be significantly better than standard therapy with four drugs given for 6 months (when new agent is used in combination for desired length of administration)
Clinical use in TB regimen	New agent can replace one of four drugs used in current 6-month regimen	New agent can replace two or more of four drugs used in current 6-month regimen
Drug-drug interactions	No serious drug-drug interactions with companion anti-TB medications	None including other anti-TB and anti-HIV agents
Drug-comorbid disease interactions	No serious drug-comorbid disease state interaction (e.g., thiacetazone and HIV)	None

* This product profile for a new anti-TB drug focuses on what would be claimed in a product label. The product profile provides broad definitions of clinically important product characteristics that do not limit or impede the unique characteristics or specific development processes of a new chemical entity.

INH and RMP. Although patients with TB caused by INH-monoresistant organisms respond well to standard treatment, those with MDR-TB do not.[64] MDR-TB patients must be treated with a combination of second-line drugs that are significantly more expensive and much more toxic and less effective than the drugs used in standard therapy.[65] When outbreaks of MDR-TB occurred in New York City in the early 1990s, the epidemic was reversed through a combination of effective control practices and systematically provided second-line anti-TB drugs.[66] However, it remains to be seen whether this strategy will be effective in stemming the MDR-TB epidemic in the Russian Federation and elsewhere.[67]

As compelling as the argument may be that new drugs are needed for MDR-TB, it is also likely that any new class of drug that improves the treatment of patients with drug-susceptible tuberculosis would be quite useful in treating those with MDR-TB. Entirely novel compounds would likely have unique mechanisms of action, so that cross-resistance with existing drugs would not be expected. However, new drugs alone will not control MDR-TB. Introducing new drugs into a poorly run programme only accelerates the development of resistance to the new compounds.

3.5.3   Improving LTBI treatment

The need for new drugs to improve the treatment of latent tuberculosis infection has received little attention until recently. It is estimated that among the approximately 2 billion persons throughout the world with LTBI, between 100 million and 200 million will develop active disease during their lifetime. Of all factors that promote the progression of LTBI to active tuberculosis, HIV infection is the most important.[68] In some areas of sub-Saharan Africa, as much as 25% of the population is coinfected

with tuberculosis and HIV, and TB rates have increased dramatically in these areas.[3]

In North America and several other low-incidence countries, INH has been used to treat LTBI in persons at greatest risk of disease progression. Isoniazid also has been shown to be effective in persons with tuberculosis and HIV coinfection.[69] However, this intervention has significant limitations. Studies of LTBI treatment in patients with silicosis suggest that certain sterilizing drugs, particularly the rifamycins, are likely to be the most useful of current drugs.[70] Other sterilizing drugs, such as PZA, might be less effective if they are inactive in the continuation phase of the treatment of pulmonary TB.

Improved treatment of high-risk persons with LTBI has been deemed essential to eliminating tuberculosis in low-incidence countries such as the United States.[71] However, this improvement will not be possible without new drugs that eliminate dormant organisms. A new drug that is useful for the treatment of active disease might also have the potential for activity in latent tuberculosis, but a better understanding of the physiologic and physical state of the bacteria is needed to confirm this hypothesis.

3.6 Current status of TB drug development

Although few truly novel compounds to treat TB have been introduced into clinical practice in the past 30 years, some promising work has been done on the following classes of drugs:

- Long-acting rifamycins (e.g. rifapentine, rifabutin, rifalazil)[10–12]
- FQ compounds (e.g. levofloxacin, moxifloxacin, gatifloxacin)[13–15]
- Oxazolidinone compounds[16]
- Nitroimidazopyrans.[17]

These drug classes might provide the best means for rapidly improving TB treatment. In addition, several drug discovery programmes for TB are being conducted within the academic, government, and private sectors. This section discusses these areas of progress.

3.6.1 Progress in chemotherapy

The most significant recent progress in TB chemotherapy has been on long-acting rifamycins, which offer the possibility of more widely spaced intermittent treatment and thus a reduction in the number of DOT doses. The most promising work has been on rifapentine (Aventis), which was approved for the treatment of TB in the United States in 1998.[10] However, the optimal dose of this drug and its appropriate use in persons with HIV infection have yet to be determined.[72,73] Another related compound, rifabutin (Pharmacia Corporation), was first approved for the pre-

vention of disseminated *Mycobacterium avium* complex disease in HIV-infected persons,[74] although the drug also appears to be an effective treatment for TB.[11] Because of its lower potential for induction of hepatic microsomal enzymes, rifabutin has been recommended for HIV-infected TB patients who cannot receive RMP because of drug interactions with a variety of antiretroviral agents.[75]

Another significant advance in TB drug treatment has come from the development of FQ compounds used to treat acute bacterial infections. Despite the absence of any randomized clinical trials showing the efficacy of FQs against TB, several reported series of TB patients treated with these drugs suggested their utility.[76] Consequently, drugs in this class, such as ofloxacin, are now among the preferred second-line drugs for MDR-TB. Newer compounds in this class, such as moxifloxacin (Bayer)[14] and gatifloxacin (Bristol-Myers Squibb),[15] appear to be much more active against *M. tuberculosis* than any of the currently available FQs. It is hoped that such a drug also might be useful in treating drug-susceptible TB (e.g. by increasing the activity of once-weekly regimens).

Two entirely novel classes of compounds also are of great interest as TB drugs: oxazolidinones and nitroimidazopyrans. Oxazolidinones (Pharmacia Corporation), which have been developed as broad-spectrum antimicrobials, appear to have substantial antimycobacterial activity.[16] Linezolid, the lead compound in this series, is approved for the treatment of specific acute bacterial infections, and it is possible that there will be corporate interest in studying other compounds in this class for TB treatment. Nitroimidazopyrans are drugs related to nitroimidazoles that have been studied in the past as possible TB drugs.[77] The most promising compound in this series, PA-824 (PathoGenesis Corporation, now Chiron Corporation), has a novel mechanism of action against *M. tuberculosis* and bactericidal activity comparable to that of INH.[17] Moreover, the drug also appears to be active against nonreplicating organisms, suggesting that it might be a potent sterilizing agent capable of shortening TB treatment.

Recent studies also have suggested that immunomodulators might have a future role in TB treatment. In patients with TB, the cytokine tumour necrosis factor alpha (TNFα) is thought to be associated with fever, necrosis, and weight loss. In HIV-infected patients, TB leads to excess production of TNFα, which in turn might accelerate HIV replication and hasten the development of AIDS. Thus, there has been some interest in the use of drugs that block TNFα production, such as thalidomide and pentoxifylline. Studies have shown that administration of thalidomide improves weight gain in both HIV-positive and HIV-negative TB patients.[78] Pentoxifylline has been associated with reductions in HIV RNA in HIV-infected TB patients.[79] Limited experience also suggests that cytokine therapy

might have a role in treating patients with MDR-TB. Aerosolized interferon-gamma and subcutaneous administration of interleukin-2 have been shown to have a bacteriologic effect in patients with MDR-TB,[80,81] and clinical trials of these agents are underway.

Genomics—the systematic identification of all the genes in a cell through DNA sequencing and bioinformatic analysis—also offers great potential in terms of drug target discovery and development of new antibacterial agents, and the recently sequenced genome of *M. tuberculosis* should provide a number of new targets for novel drugs.[18] For a relatively modest single investment, researchers can establish the full complement of genes present within a pathogen and compare their sequences with those of other organisms. In the case of *M. tuberculosis*, genomics research precisely identified functions for about 40% of the 4000 genes, obtained some functional knowledge for a further 20%, but gleaned no information for the remaining 40%.[18] For those genes with functional information available, investigators often identified potential drug targets on the basis of their proposed biological role or their similarity to known bacterial drug targets. However, now that more mycobacterial sequences are becoming available, it is possible to establish which genes are specific for mycobacteria in general or confined to a given species.[82] The functions encoded by these genes, if essential, could represent new, highly specific targets for chemotherapy. In contrast, a number of genes of unknown function have been found in many bacteria, and these are often referred to as the conserved hypotheticals.[83,84] Some of these have been shown to play critical biological roles and thus represent novel targets for new broad-spectrum antibiotics.[18]

3.6.2 Existing drug discovery programmes

New TB drugs in the pipeline are few and are vulnerable to commercial pressures in both small and large companies. Even for those in the pipeline, developmental activity appears minimal, as some pharmaceutical companies will not even test new anti-infective agents for anti-TB activity. The primary reason given for this is the fear that a more lucrative indication may be jeopardized by serious drug toxicity that is only recognized when drugs are given for the much longer periods required for TB treatment than to treat acute bacterial infections. Companies also might fear that promising compounds identified as TB drugs will not be approved to treat more common conditions.

Drug discovery programmes for TB are also being conducted within the academic and government sectors. To a large extent, the academic efforts are aimed at early discovery, particularly at target identification and basic definition of the relevant biochemical processes (e.g.

biochemistry underlying cell wall construction). Although there are publicly funded efforts to screen promising candidate compounds in both in vitro and in vivo models of TB infection, including a programme funded by the US National Institute for Allergy and Infectious Diseases (US NIAID), few focused efforts are aimed at developing active compounds that emerge from such programmes. There are several examples of carefully selected targets in high-throughput screens for 'hit' generation, but in most of these programmes substantial uncertainty exists regarding the resources available for hit optimization and medicinal chemistry.

Some TB drug development projects have been 'piggy-backed' onto projects intended to produce broad-spectrum agents for a wide range of bacteria. Several arguments have been put forward for pursuing broad-spectrum rather than narrow-spectrum agents:

- A carefully chosen broad-spectrum target allows for the simultaneous development of therapeutics against multiple important pathogens, maximizing the efficient use of scarce drug development resources
- Broad-spectrum agents stand a greater chance of development for diseases, such as TB, that might not financially inspire an independent programme
- Broad-spectrum agents are more likely to be produced in large scale, which might facilitate differential pricing schemes based upon maintaining profitability.

On the other hand, several counterarguments favour development of a narrow-spectrum agent:

- Most broad-spectrum agents have limited utility against *M. tuberculosis* because of the peculiar biochemical properties of the tubercle bacillus
- Demonstrated efficacy of a broad-spectrum agent against tuberculosis might formally or informally restrict its use exclusively for TB
- Widespread use of broad-spectrum antibiotics in non-life-threatening human and agricultural applications might promote the development of drug resistance[85]
- Broad-spectrum agents often disrupt normal symbiotic intestinal flora, increasing the potential for adverse side-effects.[86]

Truly broad-spectrum agents with gram-negative and gram-positive activities are extremely difficult to envision. However, development projects focusing on broad-spectrum targets produce libraries of compounds that might possess individual members that show narrow-spectrum activity profiles, particularly as regards TB where hydrophobicity is uniformly correlated with efficacy.[20] Thus, the notion of piggy-backing TB drug development onto an existing target-directed, broad-spectrum agent effort has considerable validity.

4.0   DRUG DISCOVERY AND DEVELOPMENT PROCESS

As efforts to develop a new treatment for tuberculosis improve, researchers and their sponsoring organizations should consider the process outlined in this section as they proceed through drug discovery and development. The Global Alliance for TB Drug Development believes that this process will help ensure that new compounds to treat TB will move successfully through development, receive rapid regulatory approval, and be transferred into clinical use.

4.1   Target selection

Most of the anti-TB drugs currently used arose from microbiological screens of libraries of chemical compounds or as the result of serendipitous discoveries. In 1944, STR was first shown to cure infections with certain gram-negative bacteria, and shortly after to be highly potent against *M. tuberculosis*. The chance observation[87] that nicotinamide inhibited the growth of mycobacteria in mice led to the synthesis and testing of related compounds, culminating in the potent drug INH.[88] Activity testing of new compounds was initially performed on in vitro grown organisms, then confirmed in an animal model of infection before clinical trials were undertaken with promising compounds. Subsequently, additional drugs were developed that inhibited particular features of *M. tuberculosis*, such as the biogenesis of its complex cell wall.

Ideally, antibacterial agents display bactericidal activity and target essential functions. One means of pinpointing such functions (although this has never been applied to the tubercle bacillus) is to isolate and characterize mutants with conditionally lethal defects and then to screen for inhibitors capable of generating the same effect. This screening was previously done by monitoring microbiological parameters such as growth rate or by using an in vitro assay if a suitable functional test existed.

Science has now progressed to the point where new drugs can be identified from a rational, hypothesis-driven approach or from high-throughput screening of chemical or combinatorial libraries by a variety of automated methods. In general, drug discovery programmes are aimed at proteins whose function is known to be essential to the bacterial cell. Several important criteria are used to evaluate the suitability of a candidate enzyme or protein as a drug target:

- The level of existing validation of the target
- The deduced tractability of the target
- Availability of three-dimensional structure data
- An approachable assay system that can be readily adapted to high-throughput screening technology
- Lack of mammalian homologues.

To ensure that new antibiotics inhibit functions that are highly selective for bacteria, thereby reducing potential side effects in humans, researchers must perform in silico screening of the human genome sequence to establish that no related genes or proteins will be found in the host. Similar screens can be undertaken of the genome sequences of other pathogens to enhance specificity. Among the attractive features of highly specific drugs, like INH or PZA, are avoidance of transferable drug-resistance mechanisms in bowel flora and their indiscriminate destruction—problems that have plagued certain broad-spectrum antibiotics.

Several approaches are available to determine which genes of *M. tuberculosis* are essential and thus worthy of further investigation for drug development. Genes can be efficiently inactivated by means of allelic exchange, using haploid or partially diploid hosts[89] or through conventional or sequence-tagged transposon mutagenesis.[90–92] If the corresponding protein has an assayable function (e.g. kinase activity), this function can be used as the basis of an in vitro screen to identify inhibitors of the enriched or purified enzyme. The advantage of this approach is that it can generally be automated or converted to the high-throughput format to facilitate screening of large or complex libraries. However, whole organism screens involving recombinant *M. tuberculosis* strains with reporter activity, such as luciferase or green fluorescent protein (GFP),[93,94] are often preferable as they avoid drug permeability problems. Once an active pharmacophore has been uncovered, numerous analogues can be synthesized or identified in combinatorial libraries to isolate more active derivatives. Their potency can also be evaluated using reporter assays or biochemical techniques, such as transcriptome or proteome analysis. In this way, genes that are coregulated can be uncovered whose products might also serve as potential drug targets, as they often act concertedly in the same metabolic process.

All of these approaches are considerably facilitated by the availability of the complete genome sequence. Significant progress towards establishing a transcriptome map of tubercle bacilli has been made,[95] and a detailed description of the proteome has been published.[96] In the latter work, roughly half of the 4000 polypeptides expected from the genome sequence were detected by two-dimensional gel electrophoresis of proteins derived from *M. tuberculosis* and selected BCG strains grown under a variety of conditions. Likewise, DNA microarrays are proving to be powerful tools for probing biodiversity and studying transcriptional responses.[95,97,98] Other techniques that offer promise in terms of identifying essential

genes are the use of RNA antisense constructs to selectively ablate gene expression[99] and the yeast or bacterial two-hybrid systems.[100,101] The latter method is particularly powerful for unravelling protein networks and delineating protein-protein interactions. This information can be used, in turn, to identify ligands that block the formation of protein complexes.[102] Inhibition of two or more steps in the same biochemical pathway is a potent means of restricting bacterial growth, as illustrated by the effect of sulfones on folic acid synthesis.

If the potential drug target can be obtained in sufficient quantities, its three-dimensional structure can be established by X-ray crystallography, nuclear magnetic resonance (NMR), or molecular modelling. Virtual screening of chemical libraries then can be undertaken to identify novel inhibitors in silico. These compounds are subsequently cocrystallized with the target to better understand their interactions. The resultant structural data can be used to design inhibitors that bind more stably or form irreversible chemical bonds. Structure-assisted drug design might be possible in the case of the InhA protein of *M. tuberculosis*, an enoyl-acyl carrier protein reductase involved in mycolic acid biosynthesis.[103] A series of fatty acid analogues of the InhA protein were synthesized that abolished enzyme activity.[104]

Other approaches have been suggested to identify targets of drugs that are likely to be potent sterilizing agents. An in vitro system has been developed that consists of persisting *M. tuberculosis* that are phenotypically resistant to RMP or INH. Compounds that kill such persisting bacilli are being sought by a patented screening procedure in large chemical libraries.[105] Milligram quantities of proteins encoded by TB dormancy genes, identified in the persistence screens, have been overexpressed and purified.

Candidate proteins and processes have emerged that might be critical for the continued persistence of bacteria within patients with LTBI.[19] These processes might offer a viable treatment target for these patients, but such targets have several problems. For example, these targets might not be essential for normal growth of the bacilli, and thus conventional microbiological laboratory procedures for determining resistance might not be applicable. However, these targets are approachable through conventional assay development, inhibitor design, lead optimization, and preclinical development processes, and they are an important component of future TB drug development considerations.

4.2 Identification of lead compounds

The overall objective in identifying lead compounds is to elaborate on tight binding inhibitors with good pharmacokinetic properties. In most cases, lead compounds are identified through successfully implemented high-throughput assays and surveys of chemical diversity for compounds that inhibit the target selected. The process of lead compound identification has been greatly enhanced by the advent of combinatorial chemical approaches to generating compound diversity.[20] This technology has allowed the creation of literally millions of discrete substances that can be individually assessed for their potential to inhibit the target. Each of the resulting inhibitors then represents a starting point (i.e. lead compound) whose structure is further manipulated to improve binding and other important characteristics.

The process typically starts with an identified target and a known biochemical process involving that target (e.g. enzymatic reaction, receptor binding, macromolecular interaction). An enzyme target with an approachable assay with readily available substrates is preferred but not essential. Such an assay might be converted into a high-throughput screen that can be used to screen existing chemical libraries for lead compounds that weakly inhibit the reaction. Typical assays that are preferred might involve spectrophotometric analysis of substrates oxidized or reduced by the enzyme target or substrates whose oxidation or reduction can be coupled to the target reaction. Other assays involving manipulation of radioactive substances are also amenable to high-throughput screening, as are assays involving the measurement of receptor-ligand binding by immobilization of one of the macromolecular partners. Ideally, an assay will be produced that can be formatted to screen compounds in 96- or 384-well plates. Such an assay then is used to screen a collection of discrete compounds or natural product extracts for inhibition of the desired target. Inhibitors identified through such a process typically have weak binding potential, having emerged from a screen for activity in the midmicromolar range.

Concurrent with such assay development and screening of initial compound libraries is an effort to elucidate the three-dimensional structure of the target protein. This effort involves overproduction and purification of the desired target in an appropriate surrogate host in milligram quantities. Maintenance of activity throughout this process is critical, and estimation of stability under various conditions is often helpful. Crystallization trials of such a target then ensue either alone or in combination with the natural substrate(s). Production of diffraction-quality crystals enables solution of the final complete protein structure. Mechanistic evaluation of the enzymatic reaction using various biochemical/biophysical analyses cross-fertilises such studies by identifying the active site region and residues critical to the reaction. Such studies also suggest inhibition mechanisms for lead compounds identified in primary high-throughput screens. Cocrystallization of promising lead compounds identified in the

primary screen allows for an interpretation of their precise mechanism of action, including identification of important binding interactions.

With both a three-dimensional structure and a lead compound, an iterative process is put in place wherein new inhibitors are synthesised and then subsequently cocrystallized with the target protein to confirm binding. A variety of computational approaches to docking of related molecules predict compounds that might display improved binding, which are then referred to the synthetic chemistry team for actual production. Secondary screens involving both computational predictions and actual measurements of surrogates for intestinal absorption accompany the hit-refinement process, as do some preliminary simplistic evaluations of toxicology and mutagenicity. Such a process ideally will produce a series of compounds with tight binding (nanomolar) properties and reasonable expectations of oral bioavailability.

Lead compounds can also be identified based on known inhibitors, chemical intuition, or even known drugs. All of these processes together can produce a series of lead compounds that might be suitable for further medicinal chemical manipulation to produce candidates for preclinical evaluation.

4.3 Optimization of lead compounds

Lead compounds are optimized through synthesis of related substances while maintaining the essential features of the original compound that conferred the inhibitory property. Such processes are facilitated by the knowledge of a crystal structure of the target, especially if a cocrystal of the lead compound can be obtained. Both computer simulation and trial-and-error testing are used when attempting to maximize the 'fit' of the compound into the active site of the protein.

The process of lead compound optimization has undergone a revolution with the advent of combinatorial solid-phase 'split-and-pool' chemistry.[106,107] The number of compounds produced by an individual chemist has increased exponentially in the last 10 years as a result of this technology. Combinatorial chemistry takes advantage of a proliferation of plastic resins now available for the transient attachment of chemical building blocks (i.e. beads) during synthesis. The advantages of this approach include simple purification techniques (e.g. washing the beads) and single-bead compound screening protocols. Beads are typically constructed of polystyrene or other suitable polymers that allow for a range of chemical linker groups to be covalently introduced onto the resin. Test compounds are then sequentially attached as a series of monomers, or diversity elements, onto a defined chemical scaffold. By splitting beads into separate reaction vessels for individual steps and then pooling them following each step, researchers can create complex mixtures of compounds upon resplitting and reacting with an additional series of monomers. In this way, 10 diversity elements in each of three positions (30 monomers total) on a single scaffold can be translated into 10^3 or 1000 discrete compounds. Defined activity uncovered through high-throughput screening can be translated into scaffolds wherein a hit represents one member of a library of thousands (or millions) of structural isomers.

Molecular tagging strategies involving the introduction of separate markers of chemical reactions onto reactant beads allow for the deconvolution of the chemical steps involved in synthesizing the component contained upon a single bead. Molecular tags are introduced in very low quantity simultaneously with reactants that form the bulk of the bead-attached components. In this way, the series of chemical steps responsible for the production of a specific single compound attached to a bead containing a hit in an assay can be retraced and the chemical structure predicted. Active molecules can be resynthesized using the same steps in larger scale and then chemically characterized. In addition, deconvolution data from primary screens of potential lead series can be used directly for structure-activity relationship determinations by applying a frequency test to individual monomers across a large number of hits in a given screen.

Selection of a lead compound and analogue generation generally occurs in parallel with an initial evaluation of a compound's drug-likeness and an investigation of preliminary characteristics known to be associated with successful development programmes.[108,109] This is particularly important considering the ability of combinatorial chemistry to efficiently sample three-dimensional space around a given scaffold element and optimize binding in a completely unbiased fashion. This technology allows compound optimization even in the absence of a known target or three-dimensional structure of a target. Some constraints must be applied to the design and synthesis of such libraries, but simple computer algorithms involving $\log_P$ (a calculated estimate of the octanol : water partition coefficient), polar surface area, and other physical characteristics have been successfully applied to complex library design to eliminate lead compounds with inappropriate characteristics (e.g. poor intestinal absorption).[109–111] In general, these considerations should exclude monomers that will produce large numbers of final compounds that violate 'Lipinski's rule of five.' This rule is as follows: The molecular weight should be less than 500, the number of hydrogen bond acceptors should be less than 10, the number of hydrogen bond donors should be less than 5, and the calculated $\log_P$ should be less than 5.[112] Compounds that satisfy these criteria stand a good chance of being efficiently absorbed through the intestine and therefore of being orally bioavailable. When such

constraints are placed upon sensible scaffolds for creating focused libraries of homologues of a hit compound, the result can be a very efficient lead optimization programme in the absence of structural biological information.

In general, these processes should happen simultaneously—that is, an assessment of binding affinity for a potential analogue should be coupled with an evaluation of the likelihood that such an analogue represents a viable development candidate. Such evaluations typically involve the following:

- Assessment of toxicity (on a eukaryotic cell-line initially)
- In vitro determination of the MIC of the lead compound against *M. tuberculosis*
- In silico prediction of the likelihood that the compound will enter the bloodstream following oral administration
- Evaluation of bioavailability and efficacy in animal models
- Initial measurement of the compound's serum stability using hepatic microsomal stability assays.

All of these data must be integrated with analogue generation to select a lead series that has the greatest hope of advancement to preclinical status.

Mycobacterial drugs in particular show some properties as a group that are distinct from the broad grouping of all known drugs listed in the Comprehensive Medicinal Chemistry (CMC) drug database. Many mycobacterial drugs are prodrugs—that is, they require some form of oxidative or reductive activation process that renders them highly reactive so that they interact with a lethal cellular target. For example, INH requires activation by the catalase-peroxidase *katG* to a highly reactive intermediate.[113] Pyrazinamide requires hydrolytic activation by the pyrazinamidase *pncA*.[47] The widely used second-line antitubercular ETH requires oxidative activation by the monooxygenase *etaA*.[114] Nitroaromatic compounds currently under development as TB drugs, such as metronidazole and the nitroimidazopyrans, require reductive activation to exert a toxic effect.[17]

The activation process greatly contributes to the overall complexity of the drug development process since resistance often emerges through loss of the activating enzyme rather than through target mutation. Unfortunately, loss of such activating enzymes appears to be much more frequent than target mutation, making the rapid development of resistance a critical issue in activation-dependent drugs.[85] In addition, loss of an activation pathway can sometimes have unpredictable effects on sensitivity to other related drugs (e.g. loss of the activation pathway for ETH confers simultaneous resistance to thiacetazone and thiocarlide).[114] Activation-dependent prodrugs only arise

out of whole-cell-based screens because screens with purified enzymes will not contain the required activating enzyme.

At an early stage in screening for potential drugs, it is important to introduce tests for the sterilizing activity of any compound identified. This could be done by using a model of microbial persistence (see Section 4.1) or by using the model proposed by Wallis and colleagues for testing in vitro activity on cultures changing from log phase to stationary phase growth.[115] The theoretical basis for the mode of action of a new drug also might suggest an early screen using specific in vitro or animal models, as has been done in the search for isocitrate lyase inhibitors.[116]

4.4 Development and application of drug screens

Screens employed for discovery of antibiotics may take a variety of approaches, often driven by the information and tools available. The very first antibiotics were derived from natural products; they are produced by other microorganisms as ecological defence mechanisms. Thus, penicillins and aminoglycosides were discovered first as hits in screens detecting inhibition of growth by natural products of soil or plant microorganisms. Fermentation extracts underwent activity-directed fractionation and purification to a lead compound. From this starting point, directed analogue synthesis produced thousands of congeners that were evaluated for antimicrobial activity and cytotoxicity. A group of candidates was ultimately selected for animal model efficacy and toxicology studies, pharmacokinetics, and formulations development. Clinical evaluations for safety and efficacy were conducted on the most promising candidate. Until recently, this general approach has been the standard for discovery programmes in the pharmaceutical industry and has produced the majority of the anti-infective drugs available today. But with the large chemical libraries now created using solid-phase, computer-directed chemistry (as discussed in Section 4.3), the discovery of new antibiotics takes place by several types of screening strategies:

- Growth inhibition assays
- Intracellular growth inhibition assays
- Surrogate assays of growth inhibition
- Inhibition of target biochemical function
- Binding to known structural targets
- Computational design of inhibitors to known targets.

All of these strategies are being used presently for *M. tuberculosis* drug discovery at academic centres and some biotechnology companies. As outlined in Table 2, each has advantages and disadvantages, and each has success stories.

Table 2 Screening strategies for antibiotic discovery

Assays	Advantages	Disadvantages

Growth inhibition[1–4]	Conducted at optimum physiological conditions for growth
	    Penetration of Mtb cell or ability to reach target is confirmed
	    Similar to standard of clinical microbiology susceptibility testing
	        Some automation possible
	        Virulent human isolates can be used
		Target is unknown
		    Biosafety precautions are high
		    Clumping of the organism may interfere
		    Mtb growth is slow
		    Small volume high-throughput screens may not be feasible
		    Choice of screening concentration may not reflect achievable bioavailability
		        Requires aqueous solution or solvents (DMSO)

Intracellular growth inhibition[5–10]	Mimics growth environment in natural infection
	    Demonstrates penetration of host cell membrane and vacuoles
	    Penetration of Mtb cell or ability to reach target is confirmed
		Target is unknown
		    Biosafety precautions are high
		    Clumping of the organism may interfere
		    Reproducibility is a factor (variables include MOI, extracellular organisms, host cell source and uniformity, macrophage state of activation)
		    Mtb growth is slow
		    Small volume high-throughput screens may not be feasible
		    Choice of screening concentration may not reflect achievable bioavailability
		        Requires aqueous solution or solvents (DMSO)
		        Requires cytotoxicity and solvent controls
		        Requires standardization and validation of the assay for screening
		        Requires confirmation in a growth inhibition assay for MIC

Surrogates of growth inhibition assays*[11–16]	Conducted at optimum physiological conditions for growth
	    Penetration of Mtb cell or ability to reach target is confirmed
	        Less stringent biosafety requirements
	
	Some automation possible
	    More rapid assay
	    Clumping may be less with other mycobacterial strains
	    Can point to target with genetically manipulated strains
		Surrogate strains may respond differently from virulent Mtb
		    Clumping of Mtb cells may interfere
		    Small volume high-throughout screens may not be feasible
		    Choice of screening concentration may not reflect achievable bioavailability
		        Requires aqueous solution or solvents (DMSO)
		        Requires cytotoxicity and solvent controls
		        Requires standardization and validation of the assay for screening
		        Requires confirmation in a growth inhibition assay for MIC against virulent Mtb
		        Target unknown in attenuated or surrogate mycobacteria

Inhibition of target biochemical function[17–23]	Cell-free assay (extracts or expressed gene products)
	    Target specified
	    Less stringent biosafety requirements
	    Small volume high-throughput screens and rapid results possible
	    May be amenable to large compound libraries
		Requires knowledge and availability of critical targets
		    Single target may have redundant forms with varying sensitivities
		    Penetration of Mtb cell or ability to reach target unknown
		    Assay conditions may not be physiological
		    Choice of screening concentration may not reflect achievable bioavailability
		        Requires aqueous solution or solvents (DMSO)
		        Requires standardization and validation of the assay for screening
		        Requires confirmation in a growth inhibition assay for MIC against virulent Mtb

Binding assays of inhibitors to known structural targets[15,24–28]	Cell-free assay (purified structural elements or expressed gene products)
	    Target specified
	    Less stringent biosafety requirements
		Requires knowledge and availability of critical targets
		    Single target may have redundant forms with varying sensitivities
		    Penetration of Mtb cell or ability to reach target unknown

Table 2 (continued)

Assays	Advantages	Disadvantages

	Small volume high-throughput screens and rapid results possible
	    May be amenable to large compound libraries
	        May tolerate organic solvents
		Assay conditions may not be physiological
		    Choice of screening concentration may not reflect achievable bioavailability
		        Requires standardization and validation of the assay for screening
		        Requires confirmation in a growth inhibition assay for MIC against virulent Mtb

Computational design of inhibitors to known targets[29–37]	Target specified using expressed gene products
	    Inhibitors modelled to fit tightly into the active site of target
	        Essentially no biosafety requirements
	        Extensive libraries of small, commercially available compounds exist
	            May tolerate organic solvents
		Requires knowledge and availability of critical targets
		    Requires pure, crystallized protein
		    Requires X-ray refraction to less than 2 angstroms resolution
		    Requires powerful computer resources and specialized software
		        Process of modelling inhibitors may be slow
		        Single target may have redundant forms with varying sensitivities
		        Penetration of Mtb cell or ability to reach target unknown
		        Requires biochemical confirmation of inhibition of target function
		        Requires confirmation in a growth inhibition assay for MIC against virulent Mtb

*These assays include respiration inhibition (O_2 consumption, oxidoreduction dyes), CO_2 production, vital strains, genetically manipulated strains, luciferase-based assays, attenuated strains (H37R$_a$, BCG), surrogate species (*M. aurum*, *M. smegmatis*).

4.5 Late discovery

Lead development involves the synthesis of between 200 and 300 compounds by a medicinal chemistry team. Many of these compounds are discarded because of poor efficacy in vitro. In vivo animal testing reduces the numbers further, especially when the compounds are administered orally. Compounds still 'in the race' then are ranked in terms of their efficacy, and a short list of 10 or so is established. The compounds on the short list are reduced further during late discovery, usually to a single candidate for development plus one compound as a back-up. At this stage, in cases where a compound has very promising anti-TB activity but has missing or unwanted product characteristics, the medicinal chemistry team might be able to alter the structure of the compound or the parent chemical class in such a way that the desired product characteristics are present.

'Assessing the robustness' of a short list of compounds is essential in defining and balancing a set of preferred characteristics and in eliminating compounds that might not successfully complete development. The purpose of this process is to define and balance a set of preferred characteristics that lower the risk barrier to the successful completion of a development programme by eliminating compounds that are not validated by predefined criteria. The process of validating lead compounds according to

the predefined criteria generally incorporates standard tests (that have been developed to assess key characteristics of any compound) and specific tests (that incorporate disease-specific characteristics desired in a new drug candidate). Very often, a sponsor starts the process with a previously designed checklist or menu of tests. Decisions about which tests to employ to assess the robustness of a new chemical class will be determined by a multidisciplinary group of medicinal and pharmaceutical chemists, pharmacologists, toxicologists, and patent and marketing experts.

The following issues are commonly considered in assessing the robustness of a lead compound:

- Formulation feasibility
- Pharmacokinetics; ADME; and pharmacodynamics
- Toxicology
  - Mutagenicity/genotoxicity
  - Acute toxicity
  - Subacute toxicity (5 to 7 days) and preliminary dose range finding
- Safety (secondary) pharmacology
- Intellectual property (patent) situation.

In addition, the chemical and pharmaceutical development team must begin a complex series of industry development steps. The sections that follow discuss all of these issues in detail.

4.5.1 Formulation feasibility

A pharmaceutical chemist should be able to formulate an orally bioavailable, stable finished drug product that meets all of the characteristics required of a new anti-TB compound. For a new TB product, an orally bioavailable dosage form is the 'gold standard' of drug delivery, although in the future, other delivery technologies might be used (e.g. liposomes). However, formulating a finished drug product that meets all of the characteristics required of a new anti-TB compound might not be a straightforward process. Formulation depends on the physical/chemical characteristics of a given compound (e.g. aqueous solubility, particle size if the compound is insoluble) and its compatibility with excipients contained within the finished dosage form. Any compound that cannot be made into an orally bioavailable product for TB must be evaluated with caution, unless the selected compound has extraordinary anti-TB characteristics that justify its development as a nonoral finished product.

4.5.2 Pharmacokinetics, ADME, and pharmacodynamics

The key parameters to assess are: (1) the relationship between the blood and tissue levels of the compound in comparison to its MIC/MBC against TB, and (2) the plasma or serumcidal activity of a compound against ex vivo *M. tuberculosis*. Determination of a new anti-TB compound's initial pharmacokinetic properties can sometimes be performed in parallel with assessment of its pharmacodynamic properties. Key pharmacokinetic parameters can be summed up as those required to understand the first pass metabolism in the liver and the ADME of a compound in a selected animal species. The animal species selected for initial ADME testing of the lead compound should be the same species in which the activity of the compound series was demonstrated (e.g. black mice, Swiss mice). For a new anti-TB compound, one should select a compound that can be given once a day with limited or no first pass metabolism, good oral bioavailability, and good tissue distribution (especially in the lungs).

4.5.3 Toxicology

One of the most important criteria used to assess a compound's robustness, and to make the subsequent decision on whether to continue preclinical development, is a series of critical path preclinical tests using appropriate in vitro and animal models. Commonly performed studies include mutagenicity studies, acute single dose toxicity studies, subacute (5- to 7-day) toxicity studies, and preliminary dose-ranging studies. It is important to note that, at this stage, depending on the chemical class, cost, and other factors, not all sponsors will opt to conduct preclinical testing according to International Conference on Harmonisation (ICH) Technical Requirements for Registration of Pharmaceutical Products for Human Use good laboratory practice (GLP) guidelines.[117]

Mutagenicity/genotoxicity studies are typically a series of three or four in vitro and in vivo studies that determine whether a given compound has the propensity to produce immediate mutations in test species' DNA after a relatively short exposure. The following tests are those most commonly performed and accepted by national regulatory authorities:

- Mutagenicity test on bacteria (*Salmonella typhimurium* and/or *Escherichia coli*) using the Ames technique
- Mutation assay at the thymidine kinase (TK) locus in L5178Y mouse lymphoma cells using a microtiter cloning technique
- Study of genotoxic activity using the micronucleus test in mice
- Test for chromosome aberrations by in vitro human lymphocyte metaphase analysis.

A sponsor might perform only one or two of these tests. The rest of the series can be completed during preclinical development after the best compound has been selected. Although each case is specific, positive findings (i.e. a mutation and/or genotoxicity occurred) in one of these tests would most likely result in a 'no go' decision for a given anti-TB compound.

Acute toxicity of a candidate compound is determined using two tests commonly employed in animal species: the single dose that causes death in 50% of test animals (LD[lethal dose]50) and the maximally tolerated dose (MTD)—that is, the dose at which the test animals develop nonfatal toxicity. Both tests can be conducted in rodent and nonrodent species. Depending on the compound being evaluated, these tests might need to be conducted in two species (i.e. rodent and nonrodent).

Non-GLP preliminary 5- to 7-day subacute toxicity studies are initially performed in rats, and then in a nonrodent species (e.g. dog). The results of these studies are used to assess a compound's global toxicity and to determine the dose that can be given during 1-month animal toxicology studies (i.e. the preliminary dose range).

4.5.4 Safety (secondary) pharmacology

Before a final decision can be made about the robustness of a candidate, a series of tests designed to determine a compound's secondary human pharmacology characteristics must be performed. These tests are designed to discover whether a compound has pharmacological activity against other human receptors that control

biologic functions such as heart rate, blood pressure, central nervous system, endocrine, immunologic function, and others. Ideally, the lead compound has no, or a minimal, noncritical effect on human receptors. A compound that exhibits a critical effect will receive a 'no go' decision.

4.5.5 Intellectual property (patent) protection

For the private sector to consider developing a new anti-TB compound, the compound must have relatively strong patent protection. A new previously undescribed chemical class is the basis for the strongest intellectual property protection an organization can seek. Lesser but still valuable patent protection may be gained from a 'me too' compound that is chemically unique but is based upon an already described chemical class (i.e. second medical use). Other possible but less useful intellectual property protection may be granted for products whose patent protection has expired but for which a new indication has been discovered. Intellectual property protection also may be given to a unique route of production or a unique dosage form or ratio of compounds (e.g. combination products).

4.5.6 Process development/chemistry

A major 'go/no go' criterion in any drug development programme relates to the feasibility of producing the compound in kilogram batches. Up to this point in the discovery and development process, the compound of interest has been produced (e.g. synthesized, extracted) only in gram quantities (i.e. lab scale). Therefore, a preliminary assessment of the feasibility of kilogram-batch production in a laboratory facility by chemists who specialize in industrial scale production of compounds is necessary. This step provides preliminary information on the eventual per-kilogram cost of the new compound (sponsors will be wary of developing compounds that cost more than US$1000/kg) and the potential hazards (e.g. explosive, environmental, occupational) of scaling up production of a compound. Assessing scale-up feasibility at this point is useful because it is still possible to change the route of synthesis. The developing organization also creates the delivery vehicle (e.g. oral tablet, sterile injectable, rectal suppository) ultimately used to administer the drug to humans. At this stage of development, a pharmaceutical manufacturer determines whether a compound that has shown activity in the discovery phase can feasibly be turned into a drug product.

Prior to the initiation of early development, the chemical and pharmaceutical development team produces a description of the lab scale route of synthesis (i.e. extraction or other process that yields the candidate compound). At this point, the team must be able to produce enough of the candidate compound at lab scale to conduct early development studies. Additionally, the drug substance form (e.g. salt, free base, acid) that the candidate compound will have when it is formulated into a finished drug product is determined. The team also produces an early estimate of the candidate compound's stability and a description of its physical-chemical properties (e.g. $\log_P$, enantiomers, salts, modifications). The pharmaceutical chemistry team also performs a preliminary assessment of the compound's chiral or diastereomeric purity. These same criteria also are identified for back-up compounds in case the lead compound does not go forward. The goal is to identify unresolveable issues related to physical-chemical properties of a compound that would prevent its formulation into the required dosage form.

4.6 Preclinical development

The use of any drug product in humans is based upon the premise that such an agent is safe and effective for the intended therapeutic indication. Safety and efficacy are usually demonstrated through a series of tests during the late discovery phase (see Section 4.5). Preclinical development is an extremely important and lengthy process that indicates the effects likely to be associated with the compound, such as antimicrobial activity and toxicity. Some toxicity studies often are performed during the discovery phase (see Section 4.5.3). Once a compound is selected for development, however, properly planned animal and toxicology studies must be conducted in compliance with GLP—an absolute requirement for regulatory purposes. These studies are highly controlled and documented tests to demonstrate possible effects of the compound being developed in order to define a window of safety for subsequent human clinical trials. Further, early discussions and interactions with representatives of regulatory agencies are encouraged and can provide critical guidance on the types and design of studies (both preclinical and clinical) likely to facilitate successful development of the drug.

This section discusses the animal and toxicology studies required.

4.6.1 Animal studies: the mouse model

Animal studies to test anti-TB drugs are used to assess in vivo the antimicrobial activity of a lead compound in comparison with that of existing drugs. In addition, the animal models test the compound's antagonistic, additive, or synergistic effects when given in combination with other drugs and its ability to sterilize the lesions of the experimentally infected animal. Animal models are not a substitute for clinical trials but only a part of the preclinical assessment of drugs and drug combinations.

To provide useful information, testing of anti-TB compounds should be performed in an appropriate model. Because of its exquisite susceptibility to *M. tuberculosis* infection, the guinea pig has long been the animal of choice to detect the presence of small numbers of tubercle bacilli in clinical specimens. This animal also has been used in experiments aimed at assessing the airborne transmission of tuberculosis and the impact of chemotherapy on transmission,[118] the comparative virulence of various strains of *M. tuberculosis*,[119] the protective value of *Mycobacterium bovis* BCG against a subsequent challenge with *M. tuberculosis*,[120] and the comparative anti-TB activity of several compounds and combinations given daily or intermittently.[56,121] However, in spite of its numerous qualities, the guinea pig has not been extensively used for testing new anti-TB compounds because of size, cost, and metabolism issues as well as its high susceptibility to concurrent infections.

Other species, such as the dog, rat, rabbit,[122] or monkey,[123] have been or are being used. The dog and rat are basic models for toxicologic investigations, and the rabbit has been used extensively for studying the mechanism of immunity in tuberculosis—that is, the nature and relative efficacy of acquired and native resistance to TB.[124] None of these species, because of size, cost (supply and maintenance), and ethical reasons (especially for the dog and monkey), is routinely used for experimental chemotherapy of mycobacterial diseases.

Because of its ease of handling in terms of size, supply, maintenance, robustness, and reproducibility, the mouse is the model of choice for TB.[21,22,125–127] However, the mouse develops less hypersensitivity to *M. tuberculosis* than the guinea pig and the monkey, and the course of the disease that follows experimental infection with *M. tuberculosis* in the mouse is different from that in humans. For example, the caseation process is limited in mice to the first 100 to 200 days and does not result in cavity formation with a large bacillary population. If infected with a high number of *M. tuberculosis* CFU by the intravenous route followed by 2 weeks without treatment, the mouse harbours a bacillary population that is similar in number and in metabolic state to that present in the lung cavity of human TB. Thus, when used with care, the mouse model is able to reproduce bacteriologic conditions close to those present in the natural human disease and provide information on compound activity that can be extrapolated to humans.

The animal studies should assess bacterial burden, mortality, and organomegaly in lung tissue at baseline; during therapy; at the end of therapy; and post-therapy to assess relapse, postantibiotic effect, and development of resistance. They should include single and combination drug evaluations to better identify the place for a new TB drug within the established therapeutic regimens.

Basic requirements of the mouse model

Among the various strains of mice that have been proposed for the experimental chemotherapy of TB, the most frequently used is the common laboratory outbred 'Swiss' mouse (CD1). The differences in the immune status among outbred Swiss mice also exist among humans, and a drug or regimen that is active against the mycobacteria in the Swiss mouse is likely to be active in humans, despite the natural differences in response between individuals. However, to compensate for individual variations, a sufficient number of mice should be used.

Because the aim of experimental chemotherapy is to obtain results that can be extrapolated to human beings, there is no absolute need to use an inbred mouse strain (e.g. Balb C, C57 Bl/6, C3H), although the variability within the group would be smaller. This does not mean that inbred mice have no place in the experimental chemotherapy of TB. On the contrary, inbred mice are ideal for comparing the anti-TB activities of several compounds. By reducing variation between individual mice, fewer animals are needed. In addition, inbred mice are required for assessing the role of the immune background in the results of chemotherapy.[128]

To test the role of immunodeficiency in the response of *M. tuberculosis* to chemotherapy and the consistency of the results, immunodeficient mice, such as athymic nude mice, can be used.[129] These mice must be kept in completely sterile conditions in negative-pressure isolators. Being deprived of activated T-lymphocytes, these mice cannot control the infection and, without effective drug therapy, die rapidly of overwhelming TB.

Mice of either sex are suitable for experimental chemotherapy, but long-term experiments should use only female mice because males frequently fight with and sometimes kill each other. To have more reproducible results, infected mice should be the same age, preferably young (i.e. 4 weeks old) and not fully immunocompetent.

The strain of tubercle bacilli used in the mouse model should be well-characterized with standard virulence and drug susceptibility. The strain of choice is the H37Rv strain of *M. tuberculosis*, which maintains virulence through regular passages in the mouse. Other well-characterized strains such as Erdman (ATTC 35801) may be used provided that their virulence is maintained through regular passages in the mouse. Before infection, the strain is subcultured in Tween-80-containing media, such as Dubos Tween Albumin or Middlebrook 7H9 broths.[130] This subculture disperses the bacilli as much as possible to create a well-calibrated inoculum. After being intravenously infected with 0.1 mg wet weight (about 5×10^6 CFU) of *M. tuberculosis* H37Rv, without TB treatment at least 90% of mice die within the first month from overwhelming TB, with more than 10^8 CFU in the spleen or lungs. When the inoculum is small (i.e. 10^4 CFU or less),

mice can contain and control the infection after an initial multiplication of the organisms. The disease that follows such limited infection remains chronic[131] and nonfatal, with counts in the spleen and lungs at about 10^6 CFU.[127]

After infecting the mouse with a standard amount of tubercle bacilli, researchers determine the efficacy of a single compound or a combination by monitoring the survival/mortality rate; the evolution of body weight; the extent of gross lesions; and the enumeration of the CFU in the spleen, lungs, and liver before, during, and after the course of treatment.[132] Usually the CFU counts are performed in the spleen[133,134] or lungs[135] or both.[136,137] Spleen and lung cultures display similar overall results,[135] so either organ is appropriate for the assessment of bactericidal activity, and it is unnecessary to enumerate the CFU in both organs.

Apart from aminoglycosides, which are given subcutaneously in the upper part of the mouse back, all compounds are given orally with an oesophageal cannula (gavage) at a volume of 0.2 to 0.3 ml per mouse. These doses are administered either daily (usually six times per week) or intermittently, depending on the objective of the experiment. For oral administration, the compounds are usually dissolved or suspended and then diluted to the requested concentration in 0.05% agar-containing sterile distilled water. Control groups should be treated with solvent alone in parallel with other groups. The drug solutions are prepared weekly and stored at 4°C.

The ADME properties of a given compound in an experimental animal are almost always different from those in humans. Therefore, extrapolating the activity of a compound requires consideration of the pharmacokinetic differences of the compound between the species.

In general, effective dosages of compounds (in mg per kg body weight) in the laboratory animal are larger than those in humans. Although there are important exceptions to the rule, it appears that the smaller the size of the animal species, the larger the dosage required for activity. One possible reason for this is that drug metabolism correlates better with body surface area than body weight.[138] As shown in Table 3, the ratio of body surface area to body weight decreases sharply with increasing body weight. For most drugs, the equipotent (i.e. equally potent) dosage is 12 times larger in the mouse than in humans (see Table 4). As a result, for a compound given to the mouse at a dosage equipotent to that in humans, the peak serum level (C_{max}) is usually much higher and achieved earlier (T_{max}), but the half-life of disappearance of the drug from the blood ($T_{1/2}$) is usually shorter in the mouse than in humans. With equipotent dosages, the mouse and human should have a similar area under a curve (AUC) representing the concentration of the drug in the plasma or serum at various intervals after administration. A rough estimate of the AUC will guide choices of the relevant dosages for a valid

Table 3 Comparison of body weight and body surface area for several species

Species	Mean body weight (g)	Range of body surface area (cm²)	Ratio of surface area (cm²) to weight (g)
Mouse	22	65–70	2.9–3.1
Rat	250	350–400	1.4–1.6
Rabbit	2500	1600–1900	0.6–0.7
Dog	12,000	5600–6500	0.4–0.5
Human	66,000	16,000–18,000	0.2–0.3

assessment of the antimicrobial activity of a drug in the mouse.

As shown in Table 5, the data obtained from studies of the three main anti-TB drugs—INH, RMP, and PZA—exemplify the importance of taking into account the pharmacokinetic differences of drugs in man and mice. The commonly used dosages give AUCs equally favourable for the mouse and humans who are slow acetylators for INH, more favourable in the mouse than in humans for RMP, and less favourable in the mouse than in humans for PZA. Among the major anti-TB drugs, only RMP shows similar C_{max} and longer $T_{1/2}$, and therefore similar AUCs, in the mouse and in humans after administration of a 10 mg/kg dose,[133,139] suggesting that similar dosages of RMP should be used in the mouse and in humans. Table 6 shows that 50 mg/kg of the new FQ sparfloxacin in the mouse is equipotent to 200 mg daily in humans (i.e. 4 mg/kg), indicating that the equipotent dosages of sparfloxacin are about 12 times higher in the mouse than in humans. Similar conclusions can be drawn for other FQs[134,140-142] by comparing major pharmacokinetic parameters between the mouse and humans. In conclusion, the rule that equipotent dosages are an average 12 times higher in mice than in humans is not always applicable. The main pharmacokinetic parameters of a new anti-TB compound should be established before assessing its activity in the mouse.

The mouse model for testing a single compound

The first steps in evaluating the activity of a compound against *M. tuberculosis* in the mouse are: (1) to determine whether or not the compound is active; (2) if the compound is active, to measure its minimal effective dose (MED) in terms of survival rate and prevention of organ lesions; and (3) to determine whether the activity is of the bacteriostatic or bactericidal type.

The simplest and most rapid way to measure a compound's activity against *M. tuberculosis* in the mouse is to perform an experiment to determine if the compound prevents the death of infected mice. For that purpose, mice are infected intravenously with 0.1 mg (about

Table 4   Equipotent doses for several species

Species	Species ratio*	Dosage (mg/kg)					
Mouse	12	50	100	200	400	800	1600
Rat	6	25	50	100	200	400	800
Rabbit	3	12.5	25	50	100	200	400
Dog	1.8	7.5	15	30	60	120	240
Human	1.0	4–4.2	8–8.5	15–20	30–35	65–70	130–135

*Ratio of dosage in experimental species that is equipotent to the dosage for humans.

Table 5   Major pharmacokinetic parameters of anti-TB drugs

Test species	Drug dosage (mg/kg)	C_{max} (µg/ml)	T_{max} (h)	$T_{1/2}$ (h)	AUC (mg-h/litre)
		INH			
Mouse	25 mg/kg	28.20 ± 3.8	0.25 ± 0	1.7 ± 0.17	52.2 ± 2.2
Human[38]	6.2 ± 6 mg/kg				
	Rapid acetylors	5.4 ± 20	1.1 ± 0.5	1.54 ± 0.31	19.3 ± 6.1
	Slow acetylors	7.1 ± 1.9	1.1 ± 0.6	3.68 ± 0.59	48.2 ± 1.5
		RMP			
Mouse	10 mg/kg	10.58 ± 0.28	1.33 ± 0.58	7.61 ± 1.32	139.7 ± 10.7
Human[39]	10–15 mg/kg	14.91	2.84	2.46	117.93
		PZA			
Mouse	150 mg/kg	146.1 ± 13.0	0.42 ± 0.2	1.05 ± 0.14	303.8 ± 17.9
Human[40]	27 ± 4 mg/kg	38.7 ± 5.9	1 ± 0	9.6 ± 1.8	520 ± 101

Note: dosage was a single oral dose. C_{max} is peak level of drug in plasma; T_{max} is time to peak level of drug in plasma; $T_{1/2}$ is half-life of elimination; AUC is area under the serum concentration–time curve.

Table 6   Major pharmacokinetic parameters of sparfloxacin

Test species	Drug dosage (mg/kg)	C_{max} (µg/ml)	T_{max} (h)	$T_{1/2}$ (h)	AUC (mg-h/litre)
Mouse	5 mg/kg	0.25	0.3	5.0	0.74
	50 mg/kg	2.80	0.75	5.0	15.18
	100 mg/kg	8.54	0.75	5.5	47.94
Human	200 mg	0.70	4	20.8	18.75
	400 mg	1.18	5	18.2	32.73

Source: Lalande V, Truffot-Pernot C, Paccaly-Moulin A, Grosset J, Ji B. Powerful bactericidal activity of sparfloxacin (AT-4140) against *Mycobacterium tuberculosis* in mice. Antimicrob Agents Chemother 1993; 37: 407–413. Reprinted with permission of the American Society for Microbiology.

5×10^6 CFU) of *M. tuberculosis* H37Rv strain and treated once daily with various dosages for 28 days beginning the day after infection. At the end of 28 days, all surviving mice are sacrificed and autopsied to detect gross lung lesions and spleen enlargement. The results are evaluated by comparison with controls.

Two control groups of mice are required: (1) a negative control group of untreated mice, of which a great majority will die within 28 days of infection with numerous gross lung lesions and significant splenomegaly, and (2) a positive control group of mice treated with 25 mg/kg INH daily, all of which should be alive and without any organ lesions 28 days after infection. The larger the size of control and treatment groups, the easier it is to detect small differences in outcome. For statistical analysis, each group should consist of at least 30 animals.

The activity of the tested compound can be measured by several parameters: weekly increases of body weight

during treatment, mortality rate during treatment or survival rate upon completion of the 28-day treatment, and gross lung lesions and spleen enlargement of the surviving mice. Determining the MED involves determining the smallest dosage that significantly increases the survival rate and reduces the development of organ lesions among the treated mice.

By definition, a compound that reduces the mortality and the development of organ lesions in treated mice also reduces or even prevents the multiplication of *M. tuberculosis* in the infected host and has at least some bacteriostatic activity. The compound might also be able to kill *M. tuberculosis* and consequently have some bactericidal activity. The precise measurement of the antimicrobial activity relies on the evolution of CFU counts in the organs of mice before, during, and after compound administration. This measurement should be made in well-defined conditions. Compound administration should be initiated when the bacillary population in the lungs and spleen is not larger than 10^6 CFU. If the mice have much more than 10^6 CFU of *M. tuberculosis* in their lungs or spleen, drug-resistant mutants will be present before and during treatment. The number of drug-susceptible CFU will decrease and the number of drug-resistant CFU will increase, resulting in an imprecise measurement of the antimicrobial activity of the tested compound. One can circumvent this by performing total CFU counts on a plain solid culture medium and drug-resistant CFU counts on a drug-containing medium.[143]

In practice, two methods for measuring antimicrobial activity are possible: mice are infected either with 0.1 mg *M. tuberculosis* and started on treatment from the day after infection or with 0.0001 mg *M. tuberculosis* (about 5×10^3 CFU) and started on treatment 14 days later, when the bacillary population in the lungs and spleen has reached between 10^5 and 10^6 CFU. With the first method, it is possible to combine the determination of the MED with the measurement of the antimicrobial activity.

In order to mimic the limited size and metabolically inactive state of the bacillary population thought to be present in subjects with quiescent TB infection, mice are first vaccinated with 5×10^3 *M. bovis* BCG CFU and then 1 month later infected with 0.0001 mg *M. tuberculosis* (5×10^3 CFU). Because of the presence of protective immunity, the growth of *M. tuberculosis* is rapidly stopped, and a chronic disease develops with a rather stable and limited bacillary population, the size of which is around 10^5 CFU in the lungs and spleen. This model has been used to test the sterilizing activity of a single compound or combination, or the activity of a compound for preventive therapy of tuberculosis.[139,144]

An elegant mouse model to test the sterilizing properties of anti-TB drugs is the model developed by Robert M. McCune, Jr, and Ralph Tompsett at Cornell University.[145,146] The Cornell model offers a relatively rapid means to assess the sterilizing activity of a single compound or combination. Mice infected intravenously with 0.1 mg *M. tuberculosis* were started on treatment less than 20 min after infection with 18–20 mg/kg INH at 0.0125% concentration and 3500 mg/kg PZA at 2.0% concentration, mixed in the daily diet for 12 weeks. All of the mice were culture-negative at the end of treatment. After 3 more months without treatment, one-third of the animals relapsed with culture-positive spleens and lungs. The organisms were still drug-susceptible, indicating that they had persisted in a latent state in culture-negative animals. The Cornell model makes it possible to determine the ability of a single compound[147] or combination[148] given at the end of the first 100 days of treatment to prevent relapse.

A mouse model in which the lungs of mice are directly exposed to a low-dose aerosol of *M. tuberculosis* has been designed to mimic newly acquired TB in which the patient has recently converted to tuberculin positivity.[149] When placed in a special exposure chamber (Glas-col Inc., Terre Haute, Indiana, USA), the mice inhale approximately 50 to 100 bacilli into their bronchial tree and alveolar spaces. The resulting infection grows progressively for 3 weeks while the animals generate specific immunity. Without treatment, the CFU counts increase by about 2 $\log_{10}$ during the first 10 days, increase by about 3.5 $\log_{10}$ during the next 14 days, and then decrease slightly. This model is best used 12 weeks after inoculation when a stable population of organisms is present. The impact of antituberculosis therapy on the course of the infection is monitored by plating serial dilutions of organ homogenates on solid culture media.[150,151]

The mouse model for testing drug combinations

The main lesion of pulmonary TB in humans is the tuberculosis cavity that contains a large population of about 10^8 actively multiplying tubercle bacilli and a limited number of drug-resistant mutants in a proportion of 10^{-6} to 10^{-7}.[152] These mutants are selected by monotherapy,[34] but their selection is prevented by combination therapy.[153] An experimental model aimed at reproducing the bacteriologic condition of human pulmonary TB should provide a large population of actively multiplying tubercle bacilli with drug-resistant mutants. Such a model is obtained by infecting mice intravenously with 0.1 mg *M. tuberculosis* and withholding treatment for 14 days. During these first 14 days, the bacilli actively multiply to reach 10^7 to 10^8 CFU in the lung and spleen. Monotherapy begun 14 days after infection selects drug-resistant mutants, but combination therapy prevents the selection of drug-resistant mutants. The CFU counts in the lung and

spleen decrease progressively as a function of the bactericidal activity of the drug combination. For example, when mice are treated daily with a combination of 200 mg/kg STR and 25 mg/kg INH, the selection of drug-resistant mutants is prevented and drug-susceptible organisms are progressively killed.[126] By the third month of treatment, 1000 CFU are isolated from the lung, by the sixth month only 100 CFU are isolated, and by the twelfth month no or very few CFU are isolated. Thus, in the initial phase of chemotherapy, the response of *M. tuberculosis* to the combination STR and INH is similar in the mouse and in humans. If the drug combination has a sterilizing activity and treatment is continued for sufficient duration, the lungs and spleen eventually become culture-negative and the mice may be considered cured. For example, in mice infected with 0.1 mg of *M. tuberculosis* and started 14 days later on daily treatment of a combination of 25 mg/kg INH and 25 mg/kg RMP for 120 days, all organs were culture-negative after 120 days.[154] Thus, in the continuation phase of chemotherapy, the fate of *M. tuberculosis* in the tissues of mice has numerous points of similarity to humans, and the information derived from the chemotherapy of experimental murine TB is suggestive of what can be expected from treatment with the same drug combinations in humans.

The issue of the stability of the culture-negative state upon completion of treatment is addressed by keeping mice without treatment for a few months. The difference in the stability of the cure of the STR-plus-INH regimen and the RMP-plus-INH regimen is striking. After 6 months of treatment with 25 mg/kg INH and 10 mg/kg RMP, the relapse rate is 20%. This fairly high rate is quite low compared to the 75% relapse rate after 18 months of treatment with 25 mg/kg INH and 200 mg/kg STR, which was once regarded as very effective in humans. If mice are given the combination INH and RMP for 9 months, there is no relapse.[135,155,156]

One might question whether negative cultures after effective long-duration chemotherapy represent full sterilization or a latent state of infection.[146,157] This issue can be addressed by administering cortisone to mice after completion of treatment.[145,155] As shown in Table 7, administering cortisone 2 months after treatment led to 60% relapses in mice treated with INH and RMP for 9 and 12 months.

4.6.2 Toxicology studies

As stated at the beginning of Section 4.6, properly planned toxicology studies must be conducted. Some of these studies might have been conducted during the late discovery phase; however, it is essential that researchers conduct preclinical toxicology studies in compliance with good laboratory practice in order to meet regulatory requirements.

Table 7 Reactivation of experimental tuberculosis in mice by cortisone administration after completion of treatment

Drug	Treatment Duration (months)	20 mg/kg cortisone (months)	% relapses
INH + PZA	3	0	75
		1	100
INH + PZA	6	0	20
INH + RMP	6	0	20
		1	30
INH + RMP	9	0	0
		2	60
INH + RMP	12	0	0
		2	60

As discussed in Section 4.5.3, some standard tests generally reveal various types of effects. The results from these studies are used to assess the risk and determine possible target organs for adverse effects. Two animal species (rodent and nonrodent) are recommended for the GLP toxicity studies. The choice of the second animal species is an important aspect of the study, and having some common elements with humans (e.g. common metabolic profile) is usually beneficial.

Key to the development plan for a new anti-TB compound will be considerations of the therapeutic niche anticipated. Issues include patient populations targeted (e.g. native, relapse, reactivation, HIV status), disease manifestations (e.g. pulmonary, miliary), phase (e.g. initiation, continuation, prophylaxis), route of administration, duration of treatment, and the therapeutic role of the compound (e.g. substitute, addition).

Preclinical toxicology may be tested in stages, starting with short-term toxicity studies and progressing to subchronic and chronic toxicity studies. Depending upon the nature of the compound, the intended duration of clinical treatment, and the prospective type of patient, long-term carcinogenicity and reproductive toxicity studies might be required.

Although individual compounds should be considered on their own merit, the following tests are generally recommended by regulatory agencies:

- In vivo general toxicity studies:
  - Range finding study (rodent)
  - Single dose study (rodent)
  - Dose escalation toxicity study (nonrodent)
  - 4-week repeated dose toxicity study (rodent and nonrodent)
  - 13-week repeated dose toxicity study (rodent and nonrodent)
  - 6-month chronic toxicity study (rodent)
  - 9-month chronic toxicity study (nonrodent)

- Carcinogenicity studies:
  - Performed if the exposure to the new compound is planned to be at least 6 months
  - New compound administration by same route as intended for human clinical use
  - Appropriate species selection based upon pharmacology, repeat dose toxicity, metabolism, toxicokinetics, and route of administration (in absence of clear evidence favouring a particular animal species, rat is recommended)
- Reproductive toxicity studies:
  - Segment I study fertility and early embryonic development
  - Segment II study teratology
  - Segment III study peri/postnatal.

4.6.3 Process development/chemistry

Before clinical trials begin, the chemical and pharmaceutical development team continues its task of scaling up the previously determined method of drug substance production, dosage form development, and development of analytical methods to ensure that good manufacturing practices can be maintained for each batch produced. The team produces a description of the synthetic (or other production) process used to prepare the active ingredient for clinical trials at pilot plant scale (i.e. multigram- to kilogram-batch size). Included in this description is a preliminary draft of the process development report and a risk assessment of scaling up to ensure that there are no occupational or environmental hazards associated with drug production. A validated control test with preliminary specifications (i.e. certificate of analysis) describes the impurity profile and any existing degradation products of the production process and the drug substance. Data must be provided on the stability of the drug substance for at least 1 month at ambient conditions. These stability data must be provided before the drug substance can be released for early clinical trials. Also determined is the chemical structure of the drug substance produced by the pilot scale production route. These data are used to describe the analytical characteristics of the reference standard. The goals are to identify all impurities in the drug substance, provide sufficient stability data to initiate early clinical trials, and establish a drug substance reference standard.

The team produces preformulation data, including a physical-chemical description of drug substance. These data are considered in an evaluation of cost of goods for drug substance and drug product. After completing the preformulation work, the team produces a description of the proposed drug product formulation and begins the standard operating procedures (SOPs) to determine the stability and toxicology (if any) associated with the

production of the drug product. Finally, the team develops and describes an SOP for the description of the proposed drug substance packaging for early clinical trials.

4.7 Clinical trials

All clinical trials must conform with internationally accepted standards of good clinical practice (GCP). Researchers should ensure that institutional review boards and/or independent ethics committees are properly established in the countries where the research is to be conducted and that these committees have the resources and independence to review the proposed studies. Whenever possible, the control and comparison treatments should be masked and placebos used to avoid the introduction of bias for or against the new treatment. The assessment of adverse events always must be included. Documenting, monitoring, and auditing the study process is essential to ensure the quality of the data. Exceptions to the protocol and protocol errors must be documented. This section outlines the ideal procedures for testing a new anti-TB drug, including trial design, process development/chemistry, capacity and networks, laboratory issues, and surrogate markers for relapse.

4.7.1 Trial design

An underlying principle of clinical trials design is that all trials must conform with internationally accepted GCP standards. Following these guidelines ensures that the highest ethical standards are met while producing data of the highest scientific quality. The ICH adopted common guidelines for GCP in May 1996.[117] Central elements of these guidelines are procedures to ensure the protection of human subjects (consistent with the Declaration of Helsinki); clearly documented protocols specifying research objectives; patient recruitment and treatment allocation; study procedures and end points; and independent monitoring of study procedures, adverse events, and data management and analysis. Designing trials that conform to these guidelines is necessary to provide quality information needed by pharmaceutical companies for product registration and regulatory approval, and by expert advisory bodies for making recommendations on the treatment of TB.

Ethical issues in the design of clinical trials are addressed in an ICH guideline published in February 1998. The tension between the provision of the highest quality clinical care and adaptation to local standards in low-income countries is an ongoing subject of debate and discussion.[158,159] These concerns have been addressed by the world medical association in October 2000, when it revised the Declaration of Helsinki,[160] and by the US National Bioethics Advisory Commission draft guidelines on

ethical issues in international research.[161] Placebo-controlled trials are inappropriate in TB studies of active TB, and their ethical application in studies of treatment of LTBI has been questioned.[158] The control regimen should be one of the regimens recommended by a respected expert body, such as WHO or IUATLD. However, other questions, such as the provision of antiretroviral care for HIV-infected individuals and the study of therapeutic regimens that might not be immediately appropriate in the country where the study is taking place, are not so clearly answered. The best way to properly address and answer these questions is to ensure that properly constituted institutional review boards and/or independent ethics committees are established in the countries where the trial is to be conducted and that these committees have the resources and independence to review the proposed trials. Dialogue between local/national ethics committees in the country where the trial is to be conducted and the committee in the country of the sponsor of the trial might help resolve conflicts between differing ethical standards.

As noted above, the control and comparison treatments should be masked whenever possible. When the two treatments are of different lengths or different rhythms of administration, the issue of whether or not to include placebo doses to mask the patient and clinician to the regimen assignment arises. For example, if a new 3-month regimen is compared to the standard 6-month regimen, it may be necessary to include placebo treatment for the additional 3 months to evaluate the efficacy of the new regimen. However, if part of the hypothesis is that the patient's expectation of the shorter course of treatment will sufficiently enhance adherence with the new regimen, thus increasing the effectiveness of the shorter regimen, the placebo doses might not be appropriate. This decision will be driven by the hypothesis to be evaluated.

While the focus of many clinical trials is on the efficacy of a new treatment, the assessment of adverse events always must be included as an important end point. The safety of any new treatment must be carefully assessed, including interactions with any other treatments or comorbidity, particularly with HIV/AIDS. A system must be in place for immediate reporting of serious adverse events (e.g. deaths, hospitalization during the study) as should a mechanism for reviewing these events, preferably by an independent Data and Safety Monitoring Board (DSMB). These issues are dealt with in detail in an ICH guideline released in October 1994.[117]

Trials of new treatments are usually conducted in adult populations, and the data needed for rational use of drugs in paediatric populations is usually missing or woefully inadequate. As paediatricians often remark, children are not small adults, and pharmacodynamics often differ significantly between adult and paediatric populations. Also, paediatric use of a drug often requires different formulations (e.g. liquid forms, smaller tablets/capsules, lower dose tablets/capsules) than those for adults. Even taste and appearance can affect the usefulness of a drug in a paediatric population. Clinical trials of TB treatments in children are particularly important because the presentation of disease in children can be very different from that in adults. Infants with TB are more likely to have disseminated forms of the disease that are immediately life threatening. Tuberculosis meningitis is more common among children than among adults; therefore, the ability of a drug to cross the blood-brain barrier is more important with children. The ICH finalised guidelines on clinical investigations in paediatric populations in July 2000.[117]

The need for documentation, monitoring, and auditing of the trial is often viewed by investigators as the most burdensome aspect of the GCP process, yet they are essential to ensuring that quality data are obtained. The process of allocating treatments to patients (i.e. randomization) is one of the most important to document. A number of schemes have been developed,[162] all of which are designed to avoid selection bias by the investigators. Adaptive allocation schemes, which adjust the probabilities of allocation to the treatment as the trial progresses based on previous allocations and outcomes in previously enrolled patients, minimise the number of patients to be enrolled. However, these designs will not commonly be used in TB trials since individual patient outcomes will not be known for months after enrolment in the trial. In a fixed allocation scheme, the precise order in which the treatments will be allocated to patients is known before the trial begins. The use of each treatment allocation should be documented to reveal any biases in the allocation. Data forms that promote accurate recording of the information at each stage of the trial, along with procedure manuals that describe their use and provide clear definitions of the data elements to be collected, are essential.

Often overlooked is the need to record exceptions to the protocol and protocol errors. The improper allocation of treatment or some other failure to adhere strictly to the protocol occurs in almost every trial, but each instance must be clearly and completely documented to allow for an assessment of the introduction of bias. Similarly, the protocol is likely to change as trials progress and the realities of clinical practice are confronted. Changes to the protocol, along with the rationale for these changes, must be clearly documented. The ultimate objective is to allow for an external review of the trials so that regulatory bodies can be assured that they were properly conducted and that the data are of adequate quality to allow a new TB treatment to be approved.

Phase I and II trials

The first trials to be conducted in human subjects are pharmacokinetic and safety trials. These Phase I trials are conducted in HNV of either gender and are designed to give the investigator an idea of the pharmacokinetic profile and limited safety data (clinical and laboratory) on a new drug. Phase I trials can be either single- or multidose trials, but a single-dose trial is typical. The number of patients needed to conduct a Phase I trial is small; usually 15 to 30 subjects receive the new drug.

In addition to Phase I HNV trials, researchers might consider also incorporating the pharmacokinetic and safety instruments into a relatively larger (i.e. 200 to 300 patients) Phase II study that enrols patients with active TB and uses additional data instruments, such as efficacy and multiple treatment groups. This design allows a sponsor to gather more relevant data on the activity of the drug by studying a diseased population rather than an HNV population.

A critical function of Phase I/II trials is determining the optimal drug dose for the Phase III trials. One well-documented method to rapidly demonstrate drug activity in humans and to assist in selection of the optimal drug dosage is the early bactericidal activity study.[23] With EBA studies, it is difficult to repeat the 'good results' obtained from trial sites with nonimmune TB populations in trial sites with semi-immune TB populations. However, results have been consistent in sites that specialize in EBA trials, which are also the sites with nonimmune populations. It is therefore possible, with the above caveats, to determine the bactericidal activity of a new anti-TB drug as a single agent using the EBA design. The other major limitation to the current EBA trial design is its inability to detect an anti-TB drug's sterilizing activity. Section 4.7.5 provides a general discussion of an EBA trial design for studying a drug's sterilizing activity by extending the length of the EBA trial from 2 days to 5 days.

Another Phase II trial that might be considered before moving to a Phase III trial is one in which experimental regimens incorporating the new drug are studied during the first 10 weeks of treatment of newly diagnosed patients with drug-sensitive pulmonary TB. The regimens in the initial 10 weeks are designed to test the potential sterilizing activity of the new drug as well as its toxicity during the initial period when toxicity usually is apparent. After the initial 10 weeks, all patients receive a 6-month continuation phase of INH/RMP. The extra 2 months prevents any risk of treatment failure should the initial phase be suboptimal. The sterilizing activity is measured in terms of surrogate markers of relapse, the main surrogate marker being negativity of a single sputum culture at 8 weeks.[25] Other markers are also included, such as the speed of sputum conversion.

The experimental regimens allocated at random during the initial 10 weeks always contain a control regimen of the four standard drugs (INH, RMP, PZA and EMB). It might be necessary to study two or more dose sizes of the new drug, one of which would be the optimal size as indicated by the EBA study. It may also be possible to substitute the new drug for RMP or RMP and PZA in the initial 10 weeks. Other regimens might also be used if there appeared to be special reasons to do so. Each regimen should have at least 100 patients assessable at 2 months and preferably 150 to be able to measure small differences in conversion rates.

Phase III trials for active TB

The greatest challenge in the design of TB clinical trials comes with Phase III trials. These trials are usually large scale, randomized clinical trials designed to show improved or equivalent efficacy of a new treatment compared to the standard treatment among diseased patients. For TB, up to 1000 patients are enrolled in a two-arm study, treated, and then followed for TB relapse for up to 2 years, the commonly accepted primary end point for demonstrating efficacy.[24] The Phase III trial design should outline the parameters that will be used to define primary and secondary end points, including the sample sizes, confidence intervals, and statistical methods that will be used to assess the data. It is imperative that microbiologic evaluations take place at the appropriate times during the Phase III clinical trials in order to assess the true activity of the investigational agent.

Because the current drug regimen is already so effective (more than 95%), it is likely that a trial of a new drug regimen will assess whether a regimen that is shorter (less than 6 months) and/or given more intermittently (less than twice weekly in the continuation phase) is equivalent to the standard regimen. However, the statistical and analytical considerations of a trial designed to demonstrate equivalence are quite different from those of a study designed to demonstrate superiority.[163] In a superiority trial, the null hypothesis being tested is that the difference between the control treatment and the test treatment is less than a specified level (i.e. they are virtually equivalent). If the result of the trial rejects the null hypothesis, then the conclusion is that one treatment is superior to the other by at least the specified level. However, if the result of the trial does not reject the null hypothesis, one cannot automatically conclude that the treatments are equivalent. Rather, the result can be used to support only the conclusion that there were insufficient data to reject the null hypothesis. In an equivalence trial, the null hypothesis is that the control treatment and the new treatment differ by a specified level (i.e. they are not equivalent). In this case, rejecting the null hypothesis does support the conclusion that the two treatments are equivalent.

In most cases, assessing a new treatment for active TB will require an equivalence trial with a sample size that is about 15% to 20% smaller than that required for a superiority trial. For example, if a trial is designed assuming that the relapse rate with the standard TB therapy is 3.5% and that a 0% to 8.5% relapse rate in a test group is considered equivalent, then 706 patients are required for a superiority trial, with 353 in the control arm and 353 in the test arm, and 568 patients (284 in each arm) are required for an equivalence trial. The sample size for the superiority trial could be reduced to 556 patients (278 per arm) if the design were a one-tailed assessment (i.e. the null hypothesis is that the relapse rate for the new treatment is no worse that 5 percentage points higher than in the control regimen); however, failure to reject this null hypothesis is not the same as proving equivalence.

It should be noted that the sample size estimates above do not include losses to the study due to death, household moves, and other reasons. Assuming a loss rate of about 15%, about 700 to 800 patients are needed for an equivalence study using the above definitions.

Phase III trials for MDR-TB

A superiority design might be appropriate for the evaluation of the efficacy of a new treatment in a study with patients with multidrug-resistant tuberculosis. In such a study, MDR-TB regimens are defined according to susceptibility patterns as part of inclusion criteria, and patients are allocated to the control or treatment arm. Assuming that the standard regimen for MDR-TB has a 70% cure rate and that this cure rate would be improved to 80% with the introduction of the new treatment in a trial with Type I error of 5% (one-tailed) and 80% power, then about 500 patients are required (250 per arm). Changing the assumptions to a 50% cure rate with the standard MDR-TB regimen and that this rate would be improved to 70% with the introduction of the new treatment in a trial with Type I error of 5% (one-tailed) and 80% power, only 170 patients (85 per arm) are required. These trials could lead to approval of a new drug specifically for the treatment of MDR-TB in combination with other specified drugs. Useful information about drug action also might be obtained and lead to the design of an equivalence study for an approved indication for the treatment of drug-sensitive TB.

Phase III trials for latent TB

While the equivalence design requires a smaller sample size for a trial of a new treatment for active TB disease, it might require larger sample sizes for studies of latent TB infection. Assuming that the standard treatment of LTBI is 70% effective in preventing active TB in a population with a 5% risk of future disease (i.e. a 1.5% risk of disease) and that a 0.75% to 2.25% risk is considered equivalent,

a sample of 11,100 patients (5550 per arm) is needed for an equivalence trial with Type I error of 5% (one-tailed) and 80% power. A superiority trial with the same assumptions on risk but designed with the null hypothesis being that the risk in the new treatment arm is greater than 2.25% requires a sample size of 8100 patients (4050 per arm) with Type I error of 5% (one-tailed) and 80% power. Although the smaller sample size is more attractive, it must be remembered that a failure to reject this null hypothesis is not the same as proving equivalence. Again, the sample size estimates do not include losses during follow-up. Approximately 12,800 patients are needed in an equivalence trial using the above definitions.

The selection of an equivalence design also has implications for the analysis of the data. With a superiority design, the primary analysis conducted is an 'intent-to-treat' analysis. An intent-to-treat analysis means that individuals are analysed according to the treatment they were allocated regardless of whether they actually received treatment (e.g. patients might not receive the assigned treatments owing to nonadherence by the patient or a protocol error by the provider). For a superiority design, this is a conservative approach and is considered more desirable because it reflects real-life practice. In an equivalence trial, however, the intent-to-treat analysis might be more biased because anything that tends to blur the difference between the two regimens supports the conclusion that the treatments are equivalent and lead to the rejection of the null hypothesis. Therefore, an equivalence design often requires an analysis of patients treated 'per protocol'. If a new treatment is rejected in a superiority trial, a potentially better treatment might not be adopted, but no harm is done by continuing with the current regimen. If a new treatment is incorrectly determined to be equivalent, great harm could result from adopting an inferior regimen as the standard.

4.7.2 Process development/chemistry

By the time Phase II trials are beginning, pilot plant production methods should be in place and the drug product dosage form will have been determined. Before initiating larger scale clinical trials, the chemical and pharmaceutical development team continues fine-tuning the scale-up production of the drug and prepares documentation that will facilitate the transfer of the manufacturing processes from pilot scale to a pilot plant facility (multiple kilograms). The process development project manager provides a report on the scalable drug production process for the next level of production—batches used for the larger clinical trials. This report defines critical process parameters and process controls for the production of the drug substance. The chemical and pharmaceutical development team prepares the documents needed to transfer the

scale-up process to the final production site. The team also updates the validated control tests that determine impurity content and degradation products as well as the drug substance's physical and chemical characteristics. Final 1-month and 3-month interim stability data should be available, and packaging control testing will be complete.

The lab scale formulation development is also complete, and the team produces preliminary data on the final formulation (i.e. pilot scale). These data include a definition of critical process parameters and process controls for production of the drug. The team determines the acceptability of the drug product's excipients, primary packaging, vials, and rubbers/stoppers with the authorities in key countries. In addition, the team prepares the documents needed to transfer the scale-up process to the final production site.

Before initiating the pivotal Phase III clinical trials, the chemical and pharmaceutical development team continues its validation of the production processes and the transfer of these processes to the final industrial scale production (i.e. tonnes). The goal at this stage is to produce a drug with the specifications that will be included in the regulatory submission dossier.

The team develops a description of the production process for a commercial-quality drug substance and a definition of the production of starting materials, intermediaries and controls, in-process control (IPC), and specifications. Validation reports are completed for all production processes, including cleaning, as is a final report on the 3-month stability of the drug substance. The definition of the manufacturing process for the drug product is finalized, including master batch manufacturing record and a description of in-process controls available. The final formulation for Phase III clinical trials and eventual commercialization is made available, and a decision on final composition is made. Finally, control testing data on excipients (if necessary) and packaging materials are finalised.

4.7.3   Capacity and networks

Although most patients participating in a new treatment study should be enrolled in the industrial sponsor's home country, the number of patients required to demonstrate the effectiveness of a new MDR-TB drug or the similarity of a new, shorter regimen to the standard regimen likely requires recruitment of patients in countries with high TB incidence rates. Several 'middle-income' countries have large TB problems and an established research infrastructure that will support clinical trials (e.g. Brazil, India, South Africa). It is probably neither necessary nor appropriate to conduct clinical trials in poorer countries, where establishing and maintaining the necessary research infrastructure will likely be difficult. Phase IV trials to evaluate new drugs under programme conditions are appropriate in these countries (see Section 4.9.2).

One desired characteristic for establishing a clinical trial site in a particular country is that the national tuberculosis programme should be strong and steadily expanding to serve the entire country (if it does not already do so). At a minimum, the national programme should provide short course chemotherapy with directly observed treatment for RMP-containing regimens. Only a programme of this type can provide essential information, such as the annual incidence of cases by type (e.g. site of disease, smear status, drug resistance) and the prevalence of complicating comorbidity. These data allow for accurate estimates of patient enrolment in the study and whether a particular treatment is appropriate for the site. The most important comorbidity to understand is the epidemiology of HIV and AIDS. Patients who are enrolled in the trial must be tested for HIV; thus, the clinical trial site must be in an area where these testing services are available.[164] In addition, participants with HIV probably require counselling. As mentioned earlier, researchers also must consider issues surrounding treatment for HIV and for opportunistic infections associated with AIDS.

The establishment of the clinical trial site should not detract from or interfere with the national programme. Ideally, the clinical trial site will build the capacity and support the efforts of the national programme services. To the degree possible, the clinical trial activities should be integrated with the national programme activities rather than operate separately.

Another key characteristic required of a clinical trial site is that a very high proportion of enrolled patients must be able to be followed for an adequate period of time (usually 2 to 3 years for a Phase III trial). A sufficiently stable population is required so that large numbers of patients do not move out of the area; or there must be an adequately sophisticated surveillance system to track individuals within a broad region or country. The ability to ensure that patients actually receive each dose of the prescribed medication is another important criterion and usually implies directly observed treatment for every dose by a member of the study team. This is especially important when ascertaining the efficacy of a treatment is a central issue. If understanding the impact of nonadherence is of interest, the clinical trial can be designed—if supported by animal models—so that every third dose of a once-weekly regimen is omitted to simulate nonadherence, as was done in the Hong Kong study of rifapentine.[165]

Establishing a network of potential clinical trial sites is highly desirable. Individual trials require many patients, and a new drug might need to be tested in multiple regimens. A network of clinical trial sites allows an adequate number of patients to be recruited in a reasonable

period of time. Establishing a network requires coordinated advance planning to ensure that each site's capabilities are clearly understood and that certain minimum standards are met. It is probably not necessary (and possibly not even desirable), for all of the sites to have similar epidemiologic situations. The trial can be designed to account for variations in epidemiology and socio-economic factors. Provided that participants are allocated equally to each arm at each trial site, the robustness of the new treatment can be assessed.

4.7.4 Laboratory issues

Since sputum conversion and relapse after completion of treatment are the most important measures of the efficacy of TB treatment, consistent performance of laboratory services is essential. Care must be taken in collecting, labelling, and transporting specimens to ensure that they always come from the patient indicated. Collection is often best done in wide-mouthed glass or heavy plastic screw-capped tubes so that they do not leak in transit. It is more important that the technical performance of the tests be consistent than that they be particularly sensitive. The reporting system and collection of results needs careful design to avoid errors. A reference laboratory also is required for most trials, but the extent to which it duplicates the procedures carried out in the local laboratory depends on the local laboratory's standards and its capacity to do the necessary work.

Clearly stated SOPs are included in the trial protocol to ensure the quality of results and to assist in the interpretation of the data. Before starting the trial, one must carefully assess the ability to process the required number of specimens in a timely manner according to the needs of the protocol. The main quality control procedure is agreement between the local and the reference laboratories in all assessments, including positivity of direct smears, culture results, speciation of mycobacterial isolates, and drug susceptibility testing. Good quality susceptibility testing includes the checking of definitions of resistance in the running of the tests. Other quality control procedures are unlikely to be useful.

In the future, new surrogate markers (e.g. as measurement of mRNA, specific metabolic byproducts, immune markers) might be included in laboratory testing to speed the evaluation of new treatments, but this will require a great deal of technical sophistication. However, these measurements might be necessary only in the preliminary 10-week trials (Phase-II), when far fewer patients are involved than in the Phase III pivotal trials.

Sputum examination
Although they do not need refrigeration, sputum specimens must not be in transit for more than 5 days at room temperature. They are examined by direct smear for acid-fast bacilli and then cultured. Cultures are routinely tested to ensure that they are *M. tuberculosis* (or a *Mycobacterium africanum* variant in some regions of Africa). Routinely, susceptibility tests include those drugs used in the chemotherapy regimen being studied. These tests might also be conducted on the second-line drugs, which might be needed in case of a failure or relapse or serious drug toxicity necessitating a change of regimen.

Direct smears are examined after Ziehl-Neelsen staining by bright field microscopy or after staining with Auramine O by fluorescence microscopy. However, fluorescence microscopy is preferable because it is five times faster, is slightly more specific, and avoids fatigue to allow more consistent results than bright field microscopy. Cultures are tested by any conventional method and, except for pretreatment specimens, there is no advantage in obtaining results rapidly. Egg culture medium (such as the Lowenstein-Jensen medium) is commonly used as it is the most widely available and far cheaper than agar-based or liquid media. Susceptibility testing by any properly calibrated method is preferred. The protocol for the trial should usually provide that patients whose pretreatment cultures are resistant to RMP are not eligible to continue because a poor response to the regimens might be expected. Screening for RMP resistance in pretreatment cultures may therefore be done by a rapid method, such as a line probe assay for mutations in the *rpoB* gene.[166] Apart from the need to protect against RMP resistance, patients might not necessarily be excluded for drug resistance since resistance to INH and other drugs is rarely of prognostic importance. Furthermore, a comparison of responses in patients with initially drug-resistant strains and those with drug-sensitive strains might shed light on the value of the individual drugs in the regimen. However, drug-resistance exclusion policies must be carefully established according to the circumstances of each study.

The number of sputum specimens to be examined depends on their value at key times during treatment. Some key specimens also are sent to the reference laboratory. At least two, and preferably three, pretreatment specimens are collected and preserved to protect against possible contamination and to allow at least one specimen to be sent to the reference laboratory. No further specimens are collected until the second month, unless there is a need to measure the speed of sputum conversion. At 2 months, two specimens are usually collected (in case of contamination), including one for the reference laboratory. The proportion of negative cultures at 2 months is of prognostic value and is a validated surrogate marker for relapse. Further specimens might be obtained monthly during treatment (a convenient frequency to fit with

monthly clinical appraisals for toxicity and other issues); however, for evaluation of 6-month regimens, only those taken at 5 and 6 months are likely to be useful in assessing whether a failure has occurred. Ideally, because of their importance, two specimens are collected at 5 and 6 months. After treatment, sputum specimens are collected periodically over at least 18 months. Most relapses occur during the first year, but an extension to 2 years or more allows all relapses to be collected, enabling comparison with earlier studies that reported relapse rates over a 2-year and even a 5-year follow-up period. The frequency of sputum collection depends on circumstances. Ideally, monthly collections are made during the first year of follow-up, and then collections are made bimonthly or quarterly. Relapse rate is the single most important assessment; therefore, it is unwise to economize on follow-up collections. Most of the follow-up specimens are examined in the local laboratory, but a few specimens should to be sent to the reference laboratory at 6 months and at 12 months. Any positive culture obtained during follow-up is subcultured and sent to the reference laboratory for storage.

Fingerprinting pairs of pretreatment and relapse cultures from the same patient by restriction fragment length polymorphism (RFLP) typing[167] or similar methods must be done. In addition, pretreatment cultures might be needed to check on susceptibility results if there is reason to suspect a discrepancy. Therefore, the culture storage system must be carefully planned and designed. Although almost all pairs have the same RFLP pattern, a few isolated positive cultures are not due to genuine relapse and have different patterns. For example, patients who are HIV-positive might have recurrent disease due to reinfection and therefore have a different RFLP pattern. Perhaps more commonly, pretreatment isolates of only a few colonies is due to laboratory contamination. These possibilities necessitate that pretreatment cultures from all patients be stored, usually frozen at $-20°C$ with a careful record of their position within the deep-freeze. Because the space occupied by the deep-freeze is considerable and repeated electrical failures lead to death of the cultures, the reference laboratory should store pretreatment cultures. A system also must be established to send any positive relapse cultures to the reference laboratory.

Urine tests
Urine tests for drugs or their metabolites confirm that drugs are being taken during the period of treatment and not after allocated treatment should have stopped. Urine specimens are collected either when patients come for a clinic visit or at their homes. Tests for INH can be conducted with isonicotinic acid, which remains reliably positive for at least 24 h after a 100 mg dose, and for RMP using a bacteriological method.

4.7.5 Surrogate markers for relapse

The relapse rate after chemotherapy ends is an important criterion to determine the efficacy of a new treatment regimen. Relapse rate also is used as an indication of whether a new drug can improve sterilizing activity by its addition to the standard regimen or as a substitution for RMP or PZA. However, since relapse rates under random clinical trial conditions are often 3% or less, large numbers of patients are needed to demonstrate an improvement in relapse rate or even to indicate a similar rate. An improvement might be evident if the duration of the treatment period were decreased, but shortening treatment is ethically difficult without preliminary evidence that the new combination will improve sterilization. Validated surrogate markers of relapse would provide evidence on the sterilizing activity of a drug/regimen without ethical problems, without requiring large numbers of patients in a conventional clinical trial, and with a great saving in development time and cost. Regulatory bodies should recognize these markers when considering provisional registration of a new drug, even if a conventional clinical trial with a shortened treatment period is necessary for full approval.

Studies in experimental murine tuberculosis might provide a strong indication of the sterilizing activity of a new drug or a combination containing it, but human evidence is required. Studies of early bactericidal activity during the first 2 days of treatment provide valuable evidence on the activity of a drug to kill rapidly growing organisms in tuberculous cavities, but they do not measure the sterilizing activity against persisters. Surrogate markers for sterilizing activity are listed in Table 8. The most useful method for studying a drug's sterilizing activity is to determine the proportion of patients who have a negative culture of a single sputum specimen at 2 months after the start of treatment with an experimental regimen compared with the proportion on a standard regimen.[25] This method has shown excellent correlations with relapse rates in eight clinical trials, seven of which were conducted under BMRC auspices.[168] The regimens being compared can be given over the entire treatment period or only for the first 2 months, provided that an identical regimen is given to both arms in the continuation phase. Less satisfactory correlations were obtained between sputum negativity at 1 month when the proportions were nearer 50% than the 80% to 90% at 2 months.

Other surrogate markers require further study and validation. The rate of sputum conversion, which conventionally is taken as the mean or median period of sputum conversion, is less well established.[169] Since the mean time is approximately 1 month (when 50% have converted), the association with relapse is likely to be less than the 2-month conversion rate. However, the 1-month rate might

Table 8 Surrogate markers for relapse rates after chemotherapy

Method	Period after start of treatment	No. of patients required to test new drug	Forecast of relapse in	
			Individual regimens	Patients ±RMP or PZA
2-month bacteriology	8 weeks	200	++	++
Rate of sputum conversion	8+ weeks	200	+	+
Alpha antigen	14 days	200	+	+
Early bactericidal activity beyond 2 days	5+ days	30	0	+

++ = high; + = moderate; 0 = low.

still have an advantage in that it depends on quantitative rather than qualitative assessments and requires more sputum examinations than does the 2-month rate. Since the 1-month rate of sputum conversion is not adequately validated and might be less efficient than the 2-month rate, it could not be used for provisional approval, although further study is merited. Bacteriological assessments have been shown to be efficient predictors of relapse among patients in several clinical trials.[170]

The assessment of sputum conversion at 2 months measures a small proportion of patients who retain a small viable bacterial population for longer than average. The same principle has been used in a new method that measures the mRNA of the *fbpB* gene of *M. tuberculosis* in sputum or the alpha antigen (85B antigen) that it produces in sputum by a sensitive enzyme-linked immunosorbent assay (ELISA) method.[26,171] Preliminary evidence suggests that the few patients on the standard four-drug therapy who continue to produce the antigen after 14 days are likely to relapse. The ELISA method also detected the effects of adding RMP to INH in a short-term EBA-type trial.[172] No other genes of the tubercle bacillus have been explored, and clearly there is scope for extending the ELISA method using the microgrid approach.

An alternative method of studying a drug's sterilizing activity might be to extend studies of early bactericidal activity beyond 2 days. This method might be a quicker and better method for comparing regimens. In a study in which the treatment period was extended to 5 days, the EBA over the 2- to 5-day period for INH fell from its very high initial value to almost zero.[23] However, the corresponding EBA for RMP maintained its moderate value over the entire 5-day period. This finding demonstrates that extending the EBA technique to 5 days and possibly beyond might allow sterilizing activity to be measured. One difficulty in using the EBA method is that it probably yields accurate results only when the patient population is drawn from a TB epidemic with low host immunity. Furthermore, the method loses precision as the treatment period is extended. However, because the EBA method offers the prospect for rapidly estimating sterilizing activity using a small number of patients, further evaluation and validation of this method is needed.

4.8 Regulatory approval

Given the lengthy development process, any delay in receiving regulatory approval will be seen by industry as an additional tax on an already limited profit potential. Regulatory uniformity among national agencies would help remove some of the current disincentives to TB drug development. This section provides information, particularly in the area of clinical trials, that can be used in developing standardized international guidelines for regulatory approval of new TB drugs. It is hoped that such guidelines will enhance the efficiency in registering new anti-TB agents while continuing to follow current national requirements that are designed to protect individuals and public health.

4.8.1 Process development/chemistry

Regulatory requirements with regard to chemistry and pharmacy (i.e. Part II of a regulatory submission) are not specific to anti-TB drug development. By implementing all of the process development/chemistry steps discussed in Sections 4.5 to 4.7, the sponsor organization should have everything required for the new drug application or marketing authorization application. The industrial scale drug substance production process for the drug substance and the drug product have been described and finalized. All drug substance specifications have been described, and 12-month accelerated drug substance stability data have been obtained. The manufacturer will have produced an expert and/or summary document to be made available to national drug regulatory authorities for the countries to which the product dossier will be submitted.

4.8.2 Demonstration of proof of concept

Proof of concept may be established using the screening assays described in Section 4.4 and Table 2. Regulatory

agencies require that the candidate drug be a defined chemical entity that has known physical characteristics, activity against human strains of *M. tuberculosis*, an acceptable selectivity for mycobacteria in comparison to mammalian cells, and a defined mechanism of action.

4.8.3 Late discovery

A sponsor claiming that a new drug is effective against drug-resistant strains of TB must have tested of the drug against a significant number of resistant strains to characterise its potential activity. The microbiological assessment of a candidate drug must include determinations of the MIC against a large spectrum of strains using established clinical laboratory methods and the MBC. These data should be based on the methodologies and strains available in the countries identified for clinical trials, as this will facilitate approval of the clinical trials. The frequency of expected resistance and the study of laboratory-induced resistant mutants also are important data that regulatory agencies will consider.

4.8.4 Preclinical development

Data supporting the potential efficacy of a new drug must include evaluations in an established animal model. From a regulatory perspective, microbiologic data should be collected from the organ system where the disease is located in humans (i.e. the lungs for TB). For example, the US Food and Drug Administration (USFDA) expects to see culture data from animal lung tissue. These data are particularly important if the candidate drug involves a new delivery system (e.g. aerosol administration), affects the immune system for disease progression, slows bacillary metabolism, or has long-term effects.

Animal studies must be carefully designed, especially when a sponsor seeks regulatory approval for an entirely new class of compounds. For example, the proposed drug solutions should be tested to show stability for the entire storage time and that they do not precipitate or degrade while being used. These data are necessary to demonstrate that the treated animals received the specified amount of the drug. In addition, regulatory agencies consider a change in bacterial burden in relevant organ systems (i.e. the lungs) to be a more accurate marker for drug efficacy than mortality and organomegaly. Studies therefore should assess bacterial burden as well as mortality and organomegaly in lung tissue at baseline; during therapy; at the end of therapy; and post-therapy to assess relapse, postantibiotic effect, and development of resistance.

Finally, the USFDA considers the development of drug resistance a regulatory issue and feels that it should be routinely assessed for all candidate tuberculosis drugs.

Microbiological data obtained from preclinical studies are likely to provide valuable information regarding the rate at which drug-resistant mutants occur. Animal studies should include combination drug evaluations to better identify the place for a new TB drug within the established therapeutic regimens. Such information also is valuable in guiding the design of Phase III clinical trials.

4.8.5 Clinical trials

Regulatory agencies require that any new drug developed for a specific disease indication must pass a series of well-controlled clinical trials conducted under ICH GCP guidelines. Clinical trials should be designed to reflect the type of product to be studied and its perceived contribution to treatment. For example, a new rifamycin compound generally would be a substitute for RMP in the standard regimen and would be compared to a RMP-containing regimen in a Phase III trial. The trial design should consider factors such as the drug's pharmacokinetic and pharmacodynamic properties (e.g. less frequent dosing for a drug with a longer half-life, shorter overall duration of treatment for a drug with increased sterilizing activity according to pilot trials and/or preclinical data). It is imperative that microbiologic evaluations take place at the appropriate times during the Phase III clinical trials in order to assess the true activity of the investigational agent. The protocol design must identify when samples will be collected for mycobacterial culture, what is defined as an adequate sample, how the samples are to be handled, and which tests will be performed on the sample. The Phase III trial design should also outline the parameters that will be used to define primary and secondary efficacy, including the sample sizes, confidence intervals, and statistical methods that will be used to assess the data.

Adequate safety information from all phases of the drug development programme must be included in the submission for regulatory approval. In general, a minimum of 500 to 1000 patients must have been exposed to the new drug at the intended dose for the planned treatment period. The safety database should include representation from racial and ethnic groups likely to receive the product, patients of both genders, and patients infected with HIV. The submitted dossier should include sufficient pharmacokinetic information to assess the likelihood of interactions with the most common concomitant therapies, including TB and non-TB drugs.

Phase I and II trials
For bactericidal anti-TB drugs, regulatory agencies generally consider Phase II EBA trials to be a well-controlled clinical trial, provided that they are conducted under GCP conditions and at a site that specializes in such trials. The

following are important considerations for regulatory agencies in evaluating a new drug's Phase II EBA trials:

- Sample size (200 to 300 patients receive new drug)
- Three-arm minimum (logarithmic dosing)
- Treatment with a single agent for 2 to 3 days
- EBA assessment for each day and cumulative
- Complete pharmacokinetic profile in a selected number of patients
- Pharmacokinetic and pharmacodynamic analysis
- Complete safety profile (lab and clinical)
- Pre- and postsusceptibility data.

All patients receiving single-agent treatment must receive the standard TB treatment after the EBA study is completed. The EBA trial also might incorporate the evaluation of unvalidated surrogate markers of anti-TB activity (e.g. mRNA).

Phase III trials
The Phase III trial design requirements create the most challenges for sponsors of new anti-TB drug development. Proving the utility of a new drug when combined with other anti-TB drugs as a substitution or an addition is time consuming, difficult and expensive. Although the EBA design might provide a methodology to demonstrate the bactericidal activity of a given drug, the sponsor must select the best method to statistically prove the value of the new drug as part of a multidrug regimen. (Note: Since multidrug therapy is required for TB, regulatory agencies might approve a new compound for use in a specific drug regimen, as was done for rifapentine in the United States.)[173]

As described in Section 4.7.1, the statistical comparison of Phase III trials relates to either a new drug's equivalence (i.e. similarity) or its superiority to the standard treatment. Because of the high success rate with standard therapy, demonstrating statistical superiority of a new drug is usually not an option. Regardless of the statistical comparison selected, the parameters should be defined prior to the initiation of the study and included in the analysis plan of the data. In addition, microbiological considerations are key for evaluations of efficacy and must play an important role when the clinical trials are being designed. If the new drug is part of a family of drugs that is currently approved for TB (e.g. RMP and rifapentine), cross-resistance will be the paramount issue for regulatory agencies. In this case, the clinical trial must test all isolates against the approved drug and the new drug to characterize the efficacy profile. Depending upon the new drug's claims (e.g. earlier clearance of TB), the study design must incorporate samples to appropriately substantiate the claims (e.g. more cultures earlier in the initial treatment phase). If the claim is for the treatment of MDR-TB, an adequate number of patients

with baseline disease due to MDR-TB strains must participate to confirm efficacy.

Another challenge for the sponsor of new drug development in obtaining regulatory approval is determining the drug's optimal use. It is difficult to determine the optimal course of therapy (e.g. 2, 4, or 6 months) and the therapy interval (e.g. once, twice, or three times per week) without performing multiple and complex large scale clinical trials. Although it might be possible and reasonable to determine the optimal use of a new drug during the Phase III trials, it is more likely that these characteristics cannot be defined until after the new drug has undergone extensive field testing (i.e. postmarketing studies or Phase IV trials).

Receiving regulatory approval for a new drug without first knowing its optimal use characteristics is possible. Regulatory agencies might issue a provisional approval of a new drug if: (1) the Phase II EBA trials indicate the drug's bactericidal activity, (2) the drug's efficacy is demonstrated during Phase III trials through a combination of traditional and surrogate markers of activity, (3) Phase III trials prove the drug's safety at the intended dose and duration levels, and (4) the sponsor submits a credible plan for the conduct of Phase IV trials to determine the optimal use of the new drug. However, the development programme should in no way compromise the regulatory cornerstones of proving the safety and efficacy of a new drug when used in combination with other anti-TB drugs.

In reviewing the Phase III trials, regulatory agencies will likely look for the following design characteristics:

- Sample size (500 to 1000 patients to receive new drug)
- Comparison of the addition (superiority) or substitution (equivalence) of the new drug against the standard 6-month short course therapy
- Primary outcome measurement:
  - 2-month sputum conversion rate or time to sputum conversion (valid indicator)
  - Initial cure at end of treatment (valid indicator)
  - Safety at the end of treatment
- Secondary outcome measurement (determined by negative sputum culture):
  - Relapse rate at 6-month follow-up (valid indicator)
  - Relapse rate at 2-year follow-up
- mRNA analysis
- Inclusion of HIV-positive and HIV-negative patients
- Inclusion of male and female, children and adults
- EBA assessment for each day and cumulative for 2 to 7 days (subset)
- Population pharmacokinetics profile
- Complete pharmacokinetics profile in subset (to evaluate drug interactions)
- Complete safety profile (lab and clinical)
- Pre- and postsusceptibility data.

With a Phase III trials plan of this type, as well as Phase I and II trials of the type described earlier in this section and an acceptable Phase IV trials programme, a sponsor will likely receive provisional approval of a new anti-TB drug.

4.8.6 *Evaluation of surrogates*

A sponsor of a new drug can use surrogates in several ways: (1) initial evaluation of a new compound, (2) pilot dose-finding studies and proof-of-concept studies, (3) early assessment of a regimen during large Phase III trials (e.g. as an interim analysis to ensure that the new treatment is not performing at an unacceptably low level), and (4) ultimately as a management tool.

To ensure that a new drug will receive regulatory approval, a sponsor must use extreme care in using surrogates. Evaluations of a proposed surrogate must be designed such that results from the surrogate can be directly correlated with results from an established method. If an investigational surrogate to be used within a clinical trial has not yet been validated or approved for clinical use, an approved/established method (e.g. culture results) must be run in parallel. Regulatory agencies rely on the established methods in approving the drug product, with surrogate data used to support these findings.

Caution should also be used in deciding what type of event/outcome the marker is a surrogate for and what the 'gold standard' is for comparison. Several response patterns early in the course of TB treatment might suggest that markers have the potential to serve as valid indicators of response; however, it is less clear what valid indicators predict longer term consequences. One approach to validating a surrogate's ability to predict long-term effects is a trial in which there are a meaningful number of failures of treatment; however, this approach is very questionable (not least from an ethical perspective), and it is highly unlikely that a sponsor could ever initiate a study with this as the expected outcome. Alternatively, an early surrogate can be compared to a more traditional measure, such as 2-month sputum conversion.

One important caveat in validating/using a surrogate is that it measures what has already happened; its ability to predict what will happen depends upon the adequacy of treatment given after the surrogate is measured. Patients who show a favourable response during induction therapy with standard therapy still might fail and/or relapse in large numbers if their continuation therapy is inadequate.

Validation issues must be addressed prior to using a surrogate as a guide to shorten therapy in a controlled clinical setting. It also should be noted that a randomised trial incorporating such an approach should use the surrogate for those patients in either group, not just those in the experimental group who demonstrate the appropriate pattern of response. This approach, which enables the

surrogate to be predictive across treatment groups, strengthens the validation of the marker. The greater the validity of the surrogate marker, the greater the chance of obtaining early regulatory approval.

4.9 Technology transfer

It is anticipated that, through the process defined by this *Scientific Blueprint*, a new TB drug with significant benefits over current medication will be developed. This section provides guidance on how to ensure that a new drug product will be introduced to and accepted by the marketplace.

4.9.1 *Moving products to market*

Technology transfer to bring new drug products to market involves product development and commercialization. Product development efforts, including patenting, describing biological activity, assessing toxicity, developing a safety profile in humans, and demonstrating clinical efficacy at the proposed dosage and mode of administration, are well-established steps of the preclinical development and clinical testing processes. Performing these studies under codes of good manufacturing, laboratory, and clinical practice enhances the technology transfer effort. For a new anti-TB drug, discovery will probably be realised through consortia or networks of scientists interacting with biotechnology companies with the capacity to provide high-throughput screening and evaluation of candidate compounds. These companies likely will globalize or form agreements with multinational pharmaceutical companies for the product development process. In an effort to help bring a new TB drug to market within 10 years (by 2010), the Global Alliance for TB Drug Development will facilitate the formation of collaborative ventures for drug development (see Section 5.2). The Global Alliance will identify partnership candidates that clearly have the potential to move a new drug from discovery to final product in the shortest period of time possible. Identifying funding mechanisms aimed at technology transfer and the building of R&D support (i.e. field sites for trials in high-prevalence countries) will be a further priority of the Global Alliance.

Although product development would appear to be the main thrust of technology transfer, commercialization efforts begin just as early. The commercialization strategy must be developed prior to clinical testing to ensure that the needs of the target market are clearly understood and taken into consideration in developing the drug product. Developing a highly effective TB drug that is too expensive for distribution in the developing world or too demanding in terms of its administration, patient acceptance, or handling requirements is purely an academic

exercise. However, one must remember that cost is relative to the anticipated effectiveness of a new product. For example, if a cure with low risk of relapse is achievable within 2 weeks, an acceptable cost would be equal to or even slightly higher than the direct and indirect costs currently associated with successful treatment outcome.

4.9.2 Postmarketing studies (Phase IV trials)

In addition to demonstrating that a new drug is effective, researchers also must determine its optimal use and operational requirements in various settings. For drugs that have been registered for use through an appropriate regulatory body, this is accomplished through postmarketing studies. These studies usually are performed in programme settings under routine treatment conditions.

Two types of postmarketing studies are required: (1) evaluations under programme conditions of new treatment regimens in comparison to locally mandated regimens, and (2) surveillance for less common adverse effects related to the new drug, including the development of drug resistance. The primary treatment outcome variable will remain the bacteriological end point; however, the first type of study must be designed to take into account biases that could arise as a result of widely differing treatment practices. This issue might have been addressed in Phase III trials, but the operational setting always presents a less than optimal environment, and drug performance under such conditions must be known. In the second type of postmarketing study, long-term impact of the new drug is measured on the basis of retreatment requirements and surveillance of drug resistance.

Finally, patient acceptance of the new drug must be objectively assessed in order to assure national programmes that changing to a new drug for TB control is financially worthwhile and in the public interest. Financial and public health improvements relative to current treatment will have a significant impact on transmission of infection and on the financial implications of managing drug-resistant disease. Economic and financial benefits of using the new drug also should be assessed.

4.9.3 Implementation, drug procurement, and accessibility

In order for technology transfer to be completed and for the new drug to be successfully implemented in national programmes, the treatment must have significant advantages over current regimens. Ideally, an improved drug will be highly efficacious, be specific for treating tuberculosis, require considerably shorter treatment periods, have a total cost that is at least similar to current best regimens, be safe, and be easy to administer. If these improvements are achieved, implementation will be easy. The main challenge then becomes one of procurement, quality control, and supply. Experience with current anti-TB medication has shown that substandard and fake preparations abound in the market.[174,175] Furthermore, drug resistance is promoted by improper use and lack of quality assurance. A model recently proposed for managing fixed-dose combination drugs can protect a new TB drug from such abuse.[176]

To generalize the use of a new drug internationally, intergovernmental organizations, NGOs, and drug manufacturers have certain responsibilities:

- WHO establishes technical norms and informs drug manufacturers of them via the IFPMA and/or the IGPA
- IFPMA and IGPA disseminate technical and other requirements to their constituent members through the appropriate channels
- Public health agencies, NGOs, and professional societies issue technical guidelines on the use of new drugs in TB treatment
- WHO should consider including any new anti-TB drug on its Essential Drugs List (EDL), and international suppliers such as UNICEF and the International Drug Association should consider placing the new drug in their catalogues of available therapeutics
- Development institutions such as the World Bank, regional development banks, and other international aid agencies and foundations should accommodate the purchase of the new drug in loan or aid agreements
- Countries establish national drug policies and regulations to suitably control the new drug. Policy and regulation development requires full coordination among the national tuberculosis programme, the national drug regulatory authority, and the national procurement office.

New TB drugs will become widely accessible and properly used only if all of these systems are sufficiently integrated and supported by strong national TB programmes with appropriate training at all levels of the health system.

5.0 OVERCOMING THE BARRIERS TO TB DRUG DEVELOPMENT

Maximizing the probability of developing cost-effective new TB drugs is best achieved by identifying and concentrating efforts where gaps exist currently in the R&D value chain. Value chain refers to the flow of activities along a given business process, in this case the activities required to research and develop a new pharmaceutical.[177] Supporting and reinvigorating areas where there are relatively few resources or activities, while facilitating or coordinating the areas with sufficient resources and infrastructure, improves the chances that a series of prospective new drug candidates will be advanced towards registration and use for TB patients.

5.1 Scientific teams and resource flows

To identify the most detrimental gaps and bottlenecks, the Global Alliance for TB Drug Development interviewed some 50 leading scientists, business people, and programme administrators in the tuberculosis field to identify the cause of the lack of activity and potential solutions. As summarized in this section and represented in Figure 1, insufficient effort is being focused on new TB treatment options at all phases of discovery and development:

- **Basic research:** Targets and compounds identified through recent basic research are not being fully exploited
- **Discovery:** Private companies are not willing to dedicate screening resources or medicinal chemists to optimizing new compounds with TB activity (lead compounds)
- **Preclinical development:** Private companies do not have an interest in preclinical TB studies, and the public sector has limited resources for the coordinated development of preclinical studies
- **Process development/chemistry:** Activities to develop appropriate manufacturing processes are inhibited by the lack of compounds available for scale-up, as well as the unwillingness of pharmaceutical companies to dedicate process chemistry resources to TB chemotherapeutics
- **Clinical trials:** Although the infrastructure for Phase I and II clinical trials is well established, Phase III trials require additional coordination, regulatory support, and funding. However, these limitations are irrelevant without promising novel compounds emerging from preclinical studies
- **Technology transfer:** Little commercialization activity is taking place because of the lack of novel compounds in development, pharmaceutical companies' pessimistic view of the TB market, and concerns about toxicity associated with long-term use.

As the previous list illustrates, the R&D gaps are due to two facts: (1) very few new drugs are in the pipeline, and (2) drug companies have not been interested in TB because the disease appears not to be a major problem in industrialized nations. However, TB does pose a major threat to all nations. The time has come to ensure that new anti-TB drugs make it through the R&D pipeline.

In the face of the significant obstacles to new TB drug development, the Global Alliance for TB Drug Development is working with several partners, including private pharmaceutical companies, to close the gaps in TB drug discovery and development. The Global Alliance's efforts will encourage creative engagement of the public and private sectors in improving the drug development process at every phase in the R&D pipeline, giving priority to the major bottlenecks that occur relatively early in the process (i.e. late discovery and preclinical research).

5.1.1 Basic research

Relative to the other areas of TB R&D, basic research, including target selection and validation, is well supported; however, this must be evaluated in the context that all parts of the TB R&D value chain are underfunded. The majority of basic research efforts are found in academia, supported by many public-sector funding agencies throughout the world. Several pharmaceutical and biotechnology firms also are dedicating resources to basic research. Most interviewees felt that sufficient and well-characterized targets currently known could justify strong discovery efforts. Although this level of activity is encouraging, most of this work appears to be striving to achieve academic and publication goals. Rewards for a product-development focus should be implemented. Encouraging researchers to move a step beyond target selection and validation to determine how their compounds could be used effectively in lead discovery is certainly a priority.

Not only could basic research benefit from more support, it is also the segment of the value chain that is least likely to be a bottleneck. Many of the currently known targets are still to be exploited fully in discovery work, and the current work in genomics will likely provide an even richer source of target information.

5.1.2 Discovery

Early discovery phases, namely assay development and screening, are moderately funded by public-sector funding agencies and some pharmaceutical and biotechnology firms. Yet, early discovery R&D could benefit from modernization. Today's assays are generally conducted with low-throughput activity screening and certainly would be more effective and efficient if modern assay development

and high-throughput screening techniques were applied. In addition, even when compounds are identified as active against TB, TB is rarely the focus of the screen. As one pharmaceutical company executive commented, 'Anti-infectives candidates [are screened] in a TB assay, and the information goes into the dossier for the compound, but it is simply to characterize the compound more thoroughly, not to find a TB lead'.

For those organizations where the objective of the screen is to identify a TB hit, there is a significant gap in late discovery, or chemical optimization, when medicinal chemists must step in. These resources are found almost exclusively in the private sector and typically are capacity constrained, even for high-priority projects. Few private organizations are willing to dedicate medicinal chemistry expertize to optimizing a TB hit. When hits are identified through publicly funded screening programmes, the public programme depends upon the private sector to move the hit forward through chemical optimization to become a lead compound, and many hits simply languish after screening.

Several interviewees from pharmaceutical and biotechnology firms were enthusiastic about receiving outside support for additional medicinal chemists dedicated to TB lead optimisation, claiming that they would make chemists available if an outside organization funded them. However, this may be unrealistic, given that the capacity constraint often results from the unavailability of medicinal chemists in the job pool, not just within a company. On the other hand, service providers can support chemical optimization. In addition, significant medicinal chemistry resources exist in developing countries, such as India and Brazil. Tapping these resources might be a cost-effective means to fill the compound optimization gap with extremely talented chemists.

5.1.3   Preclinical development

Similar to chemical optimization, the preclinical R&D phase is a relatively significant bottleneck and requires expertise generally found only in the private sector. Companies typically have not found investing in preclinical development justified for a TB indication. There is some public funding for preclinical TB studies, but the level of resources generally is considered suboptimal, partly because of a lack of expertise in the public sector and partly because TB has not been a high priority for public institutions. In the private sector, project managers shepherd compounds through the discovery and development activities. In the public sector, preclinical activities are not closely coordinated and monitored; therefore, projects languish, leading some experts to refer to this stage as the 'compound graveyard'. As with the discovery phase, the preclinical development phase requires investment by

and creative engagement of private sector biotechnology components, pharmaceutical firms, or service providers.

5.1.4   Process development/chemistry

The current lack of activity in process development/chemistry of new anti-TB drugs stems from several causes. Few leads have emerged from discovery and preclinical work. In addition, the expertise in this R&D area lies almost exclusively in large pharmaceutical firms and, as with medicinal chemistry, is often capacity constrained. Companies are rarely willing to dedicate scarce resources to TB chemotherapeutics, which are assumed to have small returns on investment. Finally, although synthesis of small quantities of a compound for early preclinical efforts is manageable, creating an efficient, cost-effective synthesis protocol for production-level quantities is a significant challenge. Improvements that address both the lack of compounds available for scale-up as well as the challenge in accessing the expertise are essential. Fortunately, as with medicinal chemistry, it might be possible to leverage the strong process chemistry resources available in developing countries.

5.1.5   Clinical trials

When considering the R&D gaps at the clinical phase, one should consider the availability of infrastructure and promising compounds. Infrastructure for Phase I and II trials is generally considered to be well established and strong, again in the context of the general underfunding for all drug development phases. Various public institutions have developed a network of sites for conducting cost-effective clinical trials, particularly in endemic countries. Phase III infrastructure is somewhat less developed in endemic countries and might require additional coordination, regulatory support, and specific funding. Several interviewees also noted that clinical trials expertise might be a scare resource, as there have been few recent trials. It is important to note that restructuring the clinical trials process, perhaps in the same manner as has been accomplished for HIV and cancer trials, might help expedite the process. Along the same lines, identifying surrogate markers might shorten the trials themselves.

In terms of promising compounds, the state of clinical trial R&D is less encouraging. Few compounds are in trials, and those being tested are either line extensions of existing families (i.e. rifamycins), indication extensions (e.g. immunotherapies), or unlikely to improve TB treatment significantly. However, the lack of activity in clinical trials reflects a lack of compounds entering this R&D phase rather than a lack of capacity or expertise to accomplish the trials. Therefore, although there are opportunities and a need for supporting the clinical trials infrastructure,

increased efforts initially should concentrate on moving promising, novel compounds out of preclinical activities and into clinical trials. Clinical trials should be viewed not as a bottleneck but as unused capacity.

5.1.6 Technology transfer

Successful drug commercialization, including prelaunch development, launch, sales and marketing, postmarketing studies, and product life-cycle management (e.g. line extensions), is generally the province of major pharmaceutical companies. Given their pessimistic view of the TB market and the lack of novel compounds in development, it is not surprising that there is little commercial activity around TB drugs. (Most patents have expired for current drugs, and the compounds therefore are manufactured and distributed by companies focused on generic markets.) As a novel compound approaches registration, success can be achieved by cooperating with companies that have the ability to commercialize these new treatments, particularly firms with existing franchises in infectious or tropical diseases. In addition, because TB is a disease with strong support from public institutions, opportunities exist for leveraging the expertise of public organizations that are positioned to commercialize drugs that benefit the public.

5.2 Priorities for action

For its mission, the Global Alliance for TB Drug Development aims to accelerate the discovery, development, and equitable distribution of new drugs that will: (1) shorten the duration of TB treatment or otherwise facilitate its successful completion, (2) be effective against MDR-TB, and/or (3) improve the treatment of latent TB infection. The ideal product would be a sterilizing agent that kills latent TB and, as a result, shortens the course of treatment. A shorter treatment, in turn, should reduce the incidence of toxic side effects and increase patient compliance, reducing the spread of the disease and the development of MDR strains. Thus, an effective new sterilizing drug would simultaneously address all three pressing drug needs.

Closing the gaps along the tuberculosis R&D pipeline requires the involvement of the public and private sectors. Figure 2 lists some of the organizations currently working in TB drug discovery and development. It should be noted that the organizations presented above do not represent all those conducting tuberculosis R&D. The Global

Alliance developed this preliminary list based on its informal survey.

As with any pharmaceutical product development project, it will be necessary to adopt a portfolio view of the prospective compounds. The probability that a single candidate will progress from discovery through registration is less than 0.5%. Therefore, the Global Alliance will distribute its support across multiple targets and mechanisms of action, among multiple partner organizations, and along multiple phases of the R&D pipeline. Distributing support is less risky than investing all support in specific scientific approaches or organizations and encourages a steadier stream of products over time.

In addition to supporting compounds at multiple phases of the R&D pipeline, the Global Alliance will strive to ensure that some early successes in this re-emerging field are demonstrated. As with any new endeavour, demonstrating results helps build momentum, credibility, and financial support. Early successes will also help attract and retain the talent necessary to pursue the Global Alliance's mission effectively. Therefore it is advantageous to identify a number of promising fast-track candidates to receive special priority. Currently, several of the quinolones and oxazolidinones appear most promising in this regard. The Global Alliance calls on funding agencies and research organizations to devote the resources needed to support these efforts.

5.3 Summary

The Global Alliance for TB Drug Development is dedicated to closing the R&D gaps. However, advances cannot be made without investment by national and international health organizations, private sector pharmaceutical and biotechnology firms, foundations, and others. Their support is needed to develop a broad portfolio of promising candidates with a special emphasis on developing fast-track and/or sterilizing drugs. Funding agencies and research organizations must devote significant resources in the short term to close the gaps in the R&D value chain and to leverage the strengths available. Fortunately, the need, the expertise, and the enthusiasm exist. By combining resources into R&D efforts to discover and develop a broad portfolio of promising candidates, the Global Alliance and its sponsors can make a vitally important contribution to improved control and the eventual elimination of tuberculosis from every country of the world.

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