Emerging Microbes and Infections (2012) 1, e9; doi:10.1038/emi.2012.21

www.nature.com/emi

REVIEW

The role of biomedical research in global tuberculosis control: gaps and challenges

A perspective from the US National Institute of Allergy and Infectious Diseases, National Institutes of Health

Christine F Sizemore, Amanda C Schleif, Jessica B Bernstein and Carole A Heilman

Tuberculosis (TB) has been a persistent public health concern for hundreds of years. Despite advances in medicine and science, eliminating this disease has been beyond our reach. Several organizations, including the National Institute of Allergy and Infectious Diseases (NIAID), part of the National Institutes of Health (NIH), have expressed their commitment to advancing biomedical research in TB in order to increase our understanding of the causative pathogen and the disease. This basic knowledge is a critical first step in the development and implementation of new therapeutics, vaccines and diagnostics. Collaboration between researchers is a key component to accomplishing this goal; product development can no longer be limited to separate programs. Rather, the interconnectedness and possible combination of interventions must be investigated. This review will discuss ongoing TB research including NIAID's role, as well as future research that is needed to improve TB control. Emphasizing the importance of coordination among researchers, funders and advocacy groups, we aim to illustrate the fact that biomedical research, and particularly basic research, is a vital part of a complementary approach to eliminating TB across the globe.
Emerging Microbes and Infections (2012) **1,** e9; doi:10.1038/emi.2012.21; published online 25 July 2012

Keywords: biomedical research; TB diagnostics; TB therapeutics; TB vaccines; tuberculosis (TB); US National Institute of Allergy and Infectious Diseases (NIAID); US National Institutes of Health (NIH)

THE CHALLENGE OF ELIMINATING TUBERCULOSIS (TB)

Despite significant advances in medical science and health care, it has been impossible to eliminate TB as a public health concern.[1] Several factors have proven advantageous to the survival of *Mycobacterium tuberculosis* (Mtb) in humans, including population growth in urban environments and the resulting close living conditions;[2,3] increasing numbers of persons co-infected with HIV who are more susceptible to developing TB;[4] and increasing numbers of drug-resistant TB cases against which standard drug regimens are failing.[1] It is noteworthy that as much as one-third of the global population is estimated to harbor Mtb and may serve as a reservoir for active disease.[5] Initially intensified by HIV/AIDS in countries where both diseases are endemic,[6] the epidemic now takes close to two million lives each year. Decades of chemotherapy under often less than optimal treatment conditions have led to the development of drug-resistant TB that complicates cure.[7] In countries with high rates of transmission and HIV co-infection, drug-resistant Mtb strains now account for an increasing portion of new TB cases.[8–11] 'The Global Plan to Stop TB 2011–2015,' issued by the Stop TB Partnership (housed at the World Health Organization (WHO)), has set ambitious goals to halve TB prevalence and death rates by 2015, and to eliminate TB by 2050.[12] (Elimination is defined as less than one case of TB per one million population per year.) This plan is also tied to the United Nation's Millennium Development Goals.[13] To accomplish these milestones, scientific and biomedical innovations such as those supported by the National Institute of Allergy and Infectious Diseases (NIAID) are desperately needed. Improvements in existing TB care programs alone are no longer sufficient.

Effectively translating biomedical research into improved patient care and new therapeutics, preventive measures, and diagnostics requires coordination among all global TB partners. Key funders, researchers and supporters of global TB research and development have recently established several frameworks for research and program implementation. For instance, the 2011 WHO/Stop TB Partnership's 'An International Roadmap for Tuberculosis Research'[14] and 'Priorities in Operational Research to Improve Tuberculosis Care and Control'[15] create an international framework to which the research agendas of major funders and supporters, as well as biomedical science as a whole can be mapped to assure that global approaches are coordinated and key gaps identified.

NIAID, part of the US National Institutes of Health (NIH), plays a critical leadership role in TB biomedical research, supporting

National Institute of Allergy and Infectious Diseases, 6610 Rockledge Drive, Bethesda, MD 20892, USA
Correspondence: AC Schleif
E-mail: schleifac@niaid.nih.gov
Received 2 May 2012; revised 22 June 2012; accepted 23 June 2012

scientists in building a solid foundation of knowledge to advance innovations in TB.[16] NIAID's research agenda complements efforts of other global funders, other US Federal agencies, as well as public–private partnerships and businesses sponsoring new product development. In 2007, NIAID published a 'Research Agenda for Multidrug-Resistant and Extensively Drug-Resistant Tuberculosis'[17] which outlines critical gaps in knowledge about the emergence, detection, prevention and cure of drug-resistant TB. The Bill and Melinda Gates Foundation published their 'Strategy Overview: Tuberculosis' in 2009,[18] enumerating goals for intensive research and development and implementation of new products, improved use of existing tools, and advocacy for funding and political commitment to improve global TB care. Most recently, the journal *Tuberculosis* published the 2012 'Tuberculosis Vaccines: A Strategic Blueprint for the Next Decade,'[19] which focuses on biomedical, product development and operational research needs that must be addressed to successfully transition vaccine candidates to the market and into public health programs. The interconnectedness and complementarity of these selected research agendas highlights the intersection between research and programmatic efforts that is crucial for closing the gaps in TB diagnosis, prevention, and treatment, and is a theme woven throughout this commentary.

Although TB has been a topic of much discussion in medical texts since the nineteenth century, significant and focused biomedical research on this disease only truly commenced in the 1990s. Many hallmarks of the disease were well documented decades or even centuries ago, but once antibiotics became available, TB was considered a manageable disease.[20] With the reduction of TB deaths in industrialized countries came diminished interest in TB research, leaving many critical questions unanswered about its pathology, pathogenesis, epidemiology and transmission. Over the past decade, however, it became apparent that existing public health strategies and healthcare tools were no longer sufficient to contain the modern global TB epidemic, and new tools would be needed. Unquestionably, a solid understanding of the science behind the disease is a critical part of developing these tools. We reached an important milestone in public health when the WHO report 'Global Tuberculosis Control 2011' identified biomedical research as a key component of the fight against TB,[1] making it clear that not only new drugs, better vaccines and improved diagnostics, but also innovative ways of combining these modalities will be necessary to reduce new cases at a rate that would drive TB into elimination.[21,22] These strategies extend beyond a focus on product development alone. Fundamental basic research will be needed to understand the detailed dynamics of host/pathogen interaction, and how this guides transmission, pathogenesis and disease resolution.[23] For instance, it is not currently understood to what extent susceptibility to infection and disease is affected by host or pathogen genetics. Gaining a better understanding of whether and how genetic diversity among Mtb strains influences their ability to infect humans and cause disease will be critical in tracking, prioritizing and intervening in TB outbreaks. Furthermore, this understanding may allow researchers to focus on strains of greatest concern when developing new drug and vaccine candidates, as well as diagnostics that can accurately differentiate TB strains.

The ultimate goal of translational science is to improve prevention, treatment and diagnosis. New strategies for product development in these areas can no longer be limited to separate programs; rather, in order to deliver the greatest benefit to patients, the interconnectedness of host-directed interventions (traditionally vaccines) and pathogen-directed interventions (traditionally drugs) must be explored and developed. For example, adding immune supportive vaccines to drug treatment for TB may improve effectiveness, shorten duration and ultimately improve tolerability of treatment. Conversely, vaccines combined with chemoprevention of limited duration may prove effective in a broader range of individuals infected with Mtb.

Guidance for discovery, development and clinical testing of new drugs, vaccines and diagnostics has been discussed at length in the recent scientific literature.[19,24–26] However, in order to establish a robust and continuous pipeline of new products, appropriate targets for intervention at several stages in the pathogenicity cycle have to be defined in both the microbe and host. Active pulmonary TB disease, the focus of many TB control programs, is also the focus of many research studies on new interventions. TB stages that are characterized by low numbers of bacteria and limited clinical symptoms, however, are also important and need to be investigated. To eliminate a transmissible respiratory disease as a public health concern, we must understand and intervene in the processes leading to active disease and address stable cure with the eradication of bacteria from patients.

PREVENTION OF TB

Prevention of TB is a multifaceted and complex issue that requires a deep understanding of several key issues. Of particular importance are the molecular and immunologic mechanisms of transmission and infection and the dynamics of latent (asymptomatic) and subclinical disease that precede the most transmissible form of TB. Quickly and accurately identifying the presence of Mtb in people with both latent and active TB is a critical first step to prevention of additional cases. Recognizing that patients are often afflicted by more than one disease, it is important to decipher how active pulmonary and other forms of TB develop in patients with healthy immune systems as well as those who are immunocompromised. Many individuals who are exposed to Mtb do not get infected, and only a fraction of those infected develop active disease.[27] The factors that govern infection and progression to active disease are not well understood, largely because the tools needed to study these phenomena are limited.[28–30] Clinical diagnosis of infected persons is currently only possible through indirect tools such as the purified protein derivative skin test or blood tests indicating prior exposure to mycobacterial antigens.[31–33] These tools do not indicate when exposure happened or whether individuals will be able to control the infection or will go on to develop TB. To study these processes, NIAID-supported scientists are developing tools that allow the detection of small numbers of mycobacteria in human specimens by interrogating the human immune system. By looking at how human immune cells respond to Mtb antigens, they are hoping to see whether the continued presence of Mtb can be differentiated from instances where infection with Mtb has been successfully cleared either by the immune system or through chemotherapy. These studies, once coupled with investigations about the growth and location of Mtb in infected individuals over time, may provide researchers with biomarkers that can differentiate bacterial presence and host responses in those who remain healthy compared with those who develop disease.[34–37]

It may appear logical to argue that every person that may be infected with Mtb should receive preventive chemotherapy. TB therapy, however, requires many months to complete and includes the potential for complications and serious side effects. The ultimate goal, rather, is to determine which persons with latent, asymptomatic infection are most likely to develop active TB disease, then provide chemotherapy or vaccines to prevent them from becoming ill and transmitting the disease to others. To assure that chemotherapeutic or preventive

strategies are available for persons at greatest risk, these strategies need to be closely coupled with adequate diagnostic tests that clearly differentiate mild or subclinical active disease from latent infection with a low chance of progression. This differentiation is particularly important, because chemopreventive drugs and regimens do not adequately treat active disease and may result in the development of drug resistance.

The success of current chemopreventive strategies is measured by its ability to reduce the number of new cases of TB within 1–2 years of treatment.[38] However, it is not known whether preventive chemotherapeutic regimens are able to eradicate Mtb from infected persons or whether they leave behind dormant bacteria that are able to reactivate later in life. To focus chemoprevention more directly on the elimination of dormant Mtb bacteria from infected persons, researchers are trying to understand whether bacterial biochemical pathways involved in latent Mtb differ from those involved in active, pulmonary TB and to develop drugs specific to each pathway.[30,39] However, even with highly focused drug treatment strategies in communities with high rates of TB transmission and high susceptibility to disease, as is the case in HIV co-infected persons, treatment of latent TB does not necessarily protect from re-infection and repeated infections may require multiple rounds of treatment. In those instances, a stronger focus on immune protection through vaccines that are effective after exposure to Mtb may offer longer lasting benefit for individual patients, and this is a concept that is currently being explored.

The current TB vaccine, called Bacille Calmette–Guerin or BCG, is given to newborns in TB endemic countries. While BCG provides reasonable protection against childhood complications of TB, it does not provide reliable protection against pulmonary TB, the most transmissible form of the disease, in adolescents or adults.[40] Hence, new vaccination approaches either aim to replace BCG or boost initial protection through BCG and provide immunity that lasts into adulthood. Alternatively, the immune responses generated by BCG may be considered as 'background immunity' for which re-priming and boosting vaccines must be developed. To properly direct the immune response, researchers are making progress in their understanding of how BCG elicits protection against childhood complications of TB, but fails to elicit long-lasting immunity against re-infection or development of disease later in life.[41–43] These studies are serving as the foundation for further research into Mtb antigens that could improve BCG efficacy or could be used later as boosters with protein-based vaccines or vectored constructs. These studies are also contributing to development of immune assays that measure desirable characteristics of novel vaccine candidates.

Development of new vaccines against TB, whether to prevent infection or prevent disease, is a complex and difficult undertaking. Scientific studies are underway to determine how Mtb avoids destruction by the human immune system and establishes latent infection. The goal of post-exposure vaccines is to identify and direct the immune system towards microbial antigens that are present during latent infection and to which the immune system would not usually respond.

TREATING TB DISEASE

Coupled with the need to rapidly identify drug-resistant Mtb in patient samples, different treatment options are needed to cure the various forms of drug-resistant TB. New combinations of novel antibiotic classes against which drug resistance has not yet developed are considered the critical next step to save patients' lives and limit the spread of drug-resistant TB. Several organizations and consortia are evaluating new combinations of experimental and existing TB drugs to arrive at more potent regimens. For example, the 'Critical Path to TB Drug Regimens Initiative,' in collaboration with stakeholders representing the TB biomedical, clinical and product development communities, is exploring regulatory pathways for drug regimens. This would be an alternative to combining drugs after they are licensed individually.[44] Currently, different combinations of first- or second-line drugs are recommended for treatment of drug-susceptible and drug-resistant TB.[45] Clinical resistance against rifampin and isoniazid—termed multidrug-resistant TB (MDR TB)—indicates that these components of first-line therapy are no longer effective and that second-line therapeutics are indicated.[46] In 2006, extensively drug-resistant strains of Mtb (XDR TB) were documented.[1,47] These XDR TB strains failed to respond to key components of first- and second-line therapeutics (rifampin, isoniazid, fluoroquinolones and any one of the injectable agents (amikacin, kanamycin or capreomycin)). Current treatment of drug-sensitive TB requires taking 4 to 5 drugs in combination for 6 months or more, while treatment for drug-resistant TB can require therapy for up to 2 years.[46] Dramatic increases in MDR and XDR TB, and documentation of strains with resistance patterns that are not defined as MDR or XDR TB, necessitates the development of new classes of antibiotics and the rapid identification and treatment of infectious patients to curb the spread of drug-resistant TB. Persistent bacterial populations, a phenomenon occurring in many bacterial species in response to antibiotic pressure, are implicated as the cause for prolonged TB treatment and various approaches are being pursued to address this problem.[48–51] For the development of new classes of antibiotics, NIAID-supported researchers and others are determining what specific biochemical pathways are most vulnerable to drug intervention in non-replicating, persisting microbes and whether treatment can be shortened when drugs are combined in innovative ways.[50,52,53] Studying low numbers of bacteria in host tissues or under metabolic conditions that induce non-replicating states is a requirement to understand the physiological processes that bacteria utilize to avoid antibiotic killing.

A different approach that is starting to generate interest in the research community is to treat TB disease in a multifactorial way, combining antimicrobial drugs with engineered vaccines or immune stimulatory molecules to engage the host immune system in the clearance of bacteria or the prevention of persistent populations. Researchers are studying the impact of antibiotics on the host immune response and whether vaccine candidates can expedite bacterial clearance.[54] Animal models are of particular importance in these studies since even low numbers of bacteria can be measured in homogenized animal tissues, and give a good initial estimate of a drug's effectiveness against Mtb.

It is expected that a combination of novel-acting drugs and an engaged host immune system may hold the key to rapid universal treatment of TB irrespective of its drug susceptibility status. Until these new treatment options are available, however, the most urgent needs are to increase treatment options for patients with drug-resistant TB and to focus on the development of molecular diagnostics that rapidly indicate whether and what type of drug resistance is present.

TOOLS TO SUPPORT NEW CLINICAL RESEARCH AND TRIALS

Translational science is primarily recognized as an important contributor to product development. However, many scientific findings that do not have direct potential to be translated into drugs, vaccines, or diagnostics can be invaluable for studying new interventions in animals and humans. As few new drugs and no new vaccines have been licensed for several decades, evaluation of products through clinical

trials is being done at the same time that assays to support regulatory processes are being developed and evaluated. Improving standard of care for TB is particularly challenging since interventions exist against which new approaches will have to be compared. Many existing drug combinations were developed decades ago. Although generally effective against TB, a better understanding of the efficacy of first- and second-line regimens, such as the 'Bangladesh Regimen,'[55] is needed to improve the standard of care and select the most appropriate comparator regimen for licensure of additive or replacement drugs, or completely new regimens. For example, individual and population data on pharmacokinetics/pharmacodynamics and drug–drug interactions, particularly with HIV-positive patients utilizing antiretroviral therapy, will help develop models to improve our understanding of the efficacy and failure of current first- and second-line drugs when used under treatment program conditions. In preparation for this, scientists are beginning to accumulate pharmacokinetic/pharmacodynamic information on several new drugs in animal and clinical studies. These data will not only contribute to baseline knowledge but may help adjust or set current standards for optimal dose. In addition, NIAID has recently made available their HIV/AIDS Clinical Trial Networks to assist in the complex testing that will be required to obtain and accumulate clinical data.[56] As previously mentioned, BCG, while not effective at preventing adult or adolescent pulmonary TB, nevertheless provides benefit to infants and also offers reasonable protection against leprosy in many countries. Therefore, trials replacing BCG for the purpose of improving protection later in life must be preceded by sufficient efficacy studies indicating that a new vaccine or vaccine/adjuvant combination will continue to provide protection for these groups of individuals while contributing to increased efficacy for preventing adult pulmonary TB. Similar to datasets needed to develop new treatment regimens and schedules, controlled data on baseline efficacy and immunogenicity of BCG needs to be generated, particularly since performance of BCG in infants has not been rigorously assessed to date. A critical research need informing both vaccine studies and clinical care alike is the identification of clear clinical definitions of Mtb infection and TB disease, particularly in infants and persons with compromised immune function. These definitions will facilitate inclusion of appropriate individuals in clinical trials and assessment of success (protection) or failure (disease) of vaccine candidates. Combined with thorough characterization of immune responses pre- and post-vaccination, case definitions are also helpful in defining what preclinical parameters should be assessed in animal models to select the most promising vaccine candidates/adjuvants and schedules. Standardized immune assays and reagents are currently being developed and will be evaluated in upcoming clinical trials. To assist in the development of preclinical and clinical tools, NIAID provides standardized reagents and vaccine and drug testing services in animal models of TB, and also provides non-clinical development and manufacturing resources to help complete data packages needed for regulatory approval of clinical trials.[57]

Tools such as preclinical animal and *ex vivo* models need to be developed in parallel with product development efforts in order to assist product developers to: demonstrate that candidate drugs and vaccines warrant clinical investigation, identify patient populations that are most appropriate to be included in these trials, and complete trials in the most efficient manner. Many of the fundamental and basic biomedical research studies currently underway have the potential to contribute to these goals. Antigens recognized by the human and animal immune system may have potential as vaccine candidates and also as biomarkers to indicate the presence of live bacteria. Biomarkers may be integrated into diagnostic tools to identify relevant patient populations, and are also useful in clinical trials to follow early response to therapy. Rapid identification of Mtb strains with different disease-causing potential may help curb epidemics and also provide opportunities for use in animal studies to ensure that vaccines and drugs are effective against diverse clades of Mtb present in endemic areas.

All approaches focused on studying Mtb during what may be called 'paucibacillary' stages of infection or disease, defined as asymptomatic infection or subclinical disease without pathological lesions, are complicated by the lack of access to human specimens that contain sufficient bacteria for study. Animal models have been used to induce paucibacillary stages of disease through pre-treatment with antibiotics, but their relevance to human disease remains unclear.[58–60] Historically, much of TB research has been driven by hypotheses generated in mouse, guinea pig, or non-human primate models without much opportunity to validate these hypotheses in humans. Through improvements in clinical study capacity in TB endemic countries, many recent programs have focused on identifying host and microbial 'biomarkers' or 'biosignatures,' which are molecular markers that characterize various stages of infection and disease. Critical to the success of identifying relevant markers of TB is access to clinical samples from diverse, longitudinal studies in human cohorts. It is important to gain an appreciation of the diversity of human TB in various high burden countries as it is influenced by co-infections and comorbidities, and to understand the diversity of genetic variants of Mtb and how they influence transmission, infection or the likelihood of progressing to active TB.

A systems biology analysis of these markers is likely to shed light on the interconnectedness of host and microbial pathways and identify new early microbial and host markers that will lead to effective diagnostics, drugs and vaccines.

ADVANCING BIOMEDICAL RESEARCH AND PUBLIC HEALTH PRIORITIES: TRANS-DISCIPLINARY COLLABORATION IS KEY

The level of innovation that will be required to truly transform the way we develop new interventions in TB necessitates that researchers, who are currently operating in separate parallel tracks, join forces to apply cross cutting science to these difficult issues. For instance, understanding molecular mechanisms driving host–pathogen interactions will aid in developing improved diagnostics, vaccines and drugs, and in identifying early markers of immune protection and treatment response. Biomarker discovery projects that focus on diagnosis, response to therapy, or immune protection will provide important information for product development. However, to add to our understanding of the complexity of TB, these studies and markers must be analyzed in combination. For this, bioinformatics tools and algorithms to create systems approaches are essential. In addition, comparative studies to define commonalities and differences in TB globally will be a crucial but difficult and resource-intensive undertaking. In an era of uncertain global economics, close collaboration between funding organizations, scientists, and other stakeholders will be required to utilize existing resources effectively, minimize duplication of effort and foster creative science. Valuable specimens from human volunteers must be analyzed and preserved in a way that provides maximum benefit for the overall understanding of TB and the translation of research findings to improve overall care and control of this disease. This will, however, require close collaboration between basic researchers, clinicians, and animal modelers in order to arrive at the

key public health and medical questions in TB and how biomedical and particularly basic research can be applied to solve them.

ACKNOWLEDGMENTS

The authors would like to thank Mamodikoe Makhene and Gail Jacobs, who contributed their time and expertise to the development of this article. Their assistance is greatly appreciated.

1 World Health Organization. *Global tuberculosis control 2011*. Geneva: WHO Press, 2011. Available at http://www.who.int/entity/tb/publications/global_report/2011/gtbr11_full.pdf. (accessed 1 February 2012).
2 Murray M, Oxlade O, Lin HH. Modeling social, environmental and biological determinants of tuberculosis. *Int J Tuberc Lung Dis* 2011; **15** Suppl 2: S64–S70.
3 Lienhardt C. From exposure to disease: the role of environmental factors in susceptibility to and development of tuberculosis. *Epidemiol Rev* 2001; **23**: 288–301.
4 Lawn SD, Churchyard G. Epidemiology of HIV-associated tuberculosis. *Curr Opin HIV AIDS* 2009; **4**: 325–333.
5 Raviglione MC, Snider DE Jr, Kochi A. Global epidemiology of tuberculosis. Morbidity and mortality of a worldwide epidemic. *JAMA* 1995; **273**: 220–226.
6 Getahun H, Gunneberg C, Granich R, Nunn P. HIV infection-associated tuberculosis: the epidemiology and the response. *Clin Infect Dis* 2010; **50** Suppl 3: S201–S207.
7 Caminero JA. Multidrug-resistant tuberculosis: epidemiology, risk factors and case finding. *Int J Tuberc Lung Dis* 2010; **14**: 382–390.
8 Cox HS, McDermid C, Azevedo V *et al*. Epidemic levels of drug resistant tuberculosis (MDR and XDR-TB) in a high HIV prevalence setting in Khayelitsha, South Africa. *PLoS One* 2010; **5**: e13901.
9 Calver AD, Falmer AA, Murray M *et al*. Emergence of increased resistance and extensively drug-resistant tuberculosis despite treatment adherence, South Africa. *Emerg Infect Dis* 2010; **16**: 264–271.
10 Zhao M, Li X, Xu P *et al*. Transmission of MDR and XDR tuberculosis in Shanghai, China. *PLoS One* 2009; **4**: e4370.
11 Nodieva A, Jansone I, Broka L, Pole I, Skenders G, Baumanis V. Recent nosocomial transmission and genotypes of multidrug-resistant *Mycobacterium tuberculosis*. *Int J Tuberc Lung Dis* 2010; **14**: 427–433.
12 The Stop TB Partnership and the World Health Organization. *The Global Plan to Stop TB, 2011–2015*. Geneva: WHO Press, 2010. Available at http://www.stoptb.org/assets/documents/global/plan/TB_GlobalPlanToStopTB2011-2015.pdf. (accessed 30 January 2012).
13 United Nations General Assembly. *United Nations Millennium Declaration*. 2000. Available at http://www.un.org/millennium/declaration/ares552e.pdf. (accessed 14 April 2012).
14 The Stop TB Partnership and the World Health Organization. *An International Roadmap for Tuberculosis Research*. Geneva: WHO Press, 2011. Available at http://www.stoptb.org/assets/documents/resources/publications/technical/tbresearchroadmap.pdf. (accessed 14 April, 2012).
15 The World Health Organization and the Stop TB Partnership. *Priorities in operational research to improve tuberculosis care and control*. Geneva: WHO Press, 2011. Available at http://www.who.int/tb/features_archive/operational_research_priorities/en/index.html. (accessed 1 February 2012).
16 Policy Cures. *G-FINDER 2011: neglected disease research and development*. Sydney: Policy Cures, 2011. Available at http://policycures.org/publications.html. (accessed 2 March 2012).
17 NIAID Tuberculosis Working Group. *NIAID Research Agenda: multidrug-resistant and extensively drug-resistant tuberculosis*. Bethesda: NIAID, 2007. Available at http://www.niaid.nih.gov/topics/tuberculosis/research/documents/mdrxdrresearchagenda.pdf. (accessed 30 March 2012).
18 Bill and Melinda Gates Foundation Global Health Program. *Strategy Overview: Tuberculosis*. 2009. Available at http://www.gatesfoundation.org/global-health/Documents/tuberculosis-strategy.pdf. (accessed 2 May 2012).
19 Brennan MJ, Thole J. Tuberculosis vaccines: a strategic blueprint for the next decade. *Tuberculosis* 2012; **92** Suppl 1: S6–S13.
20 Daniel TM. The history of tuberculosis. *Respir Med* 2006; **100**: 1862–1870.
21 Abu-Raddad LJ, Sabatelli L, Achterberg JT *et al*. Epidemiological benefits of more-effective tuberculosis vaccines, drugs, and diagnostics. *Proc Natl Acad Sci U S A* 2009; **106**: 13980–13985.
22 Lonnroth K, Raviglione M. Global epidemiology of tuberculosis: prospects for control. *Semin Respir Crit Care Med* 2008; **29**: 481–491.
23 Russell DG, Barry CE 3rd, Flynn JL. Tuberculosis: what we don't know can, and does, hurt us. *Science* 2010; **328**: 852–856.
24 Global Alliance for TB Drug Development. Tuberculosis. Scientific blueprint for tuberculosis drug development. *Tuberculosis (Edinb)* 2001; **81** Suppl 1: S1–S52.
25 Walker KB, Brennan MJ, Ho MM *et al*. The second Geneva Consensus: recommendations for novel live TB vaccines. *Vaccine* 2010; **28**:2259–2270.
26 The New Diagnostics Working Group of the Stop TB Partnership. *Pathways to better diagnostics for tuberculosis: a blueprint for the development of TB diagnostics*. Geneva: WHO Press, 2009. Available at http://whqlibdoc.who.int/publications/2009/9789241598811_eng.pdf. (accessed 22 January 2012).
27 Vynnycky E, Fine PE. The natural history of tuberculosis: the implications of age-dependent risks of disease and the role of reinfection. *Epidemiol Infect* 1997; **119**: 183–201.
28 Lawn SD, Wood R, Wilkinson RJ. Changing concepts of 'latent tuberculosis infection' in patients living with HIV infection. *Clin Dev Immunol* 2011; **2011** pii: 980594.
29 Achkar JM, Jenny-Avital ER. Incipient and subclinical tuberculosis: defining early disease states in the context of host immune response. *J Infect Dis* 2011; **204** Suppl 4: S1179–S1186.
30 Esmail H, Barry CE 3rd, Wilkinson RJ. Understanding latent tuberculosis: the key to improved diagnostic and novel treatment strategies. *Drug Discov Today* 2012; **17**: 514–521.
31 Rangaka MX, Wilkinson KA, Glynn JR *et al*. Predictive value of interferon-gamma release assays for incident active tuberculosis: a systematic review and meta-analysis. *Lancet Infect Dis* 2012; **12**: 45–55.
32 Mandalakas AM, Detjen AK, Hesseling AC, Benedetti A, Menzies D. Interferon-gamma release assays and childhood tuberculosis: systematic review and meta-analysis. *Int J Tuberc Lung Dis* 2011; **15**: 1018–1032.
33 Diel R, Loddenkemper R, Nienhaus A. Predictive value of interferon-gamma release assays and tuberculin skin testing for predicting progression from latent TB infection to disease state: a meta-analysis. *Chest* 2012 Apr 5; doi:10.1378/chest.11-3157.
34 Gideon HP, Flynn JL. Latent tuberculosis: what the host 'sees'? *Immunol Res* 2011; **50**: 202–212.
35 Walzl G, Ronacher K, Hanekom W, Scriba TJ, Zumla A. Immunological biomarkers of tuberculosis. *Nat Rev Immunol* 2011; **11**: 343–354.
36 Ottenhoff TH, Ellner JJ, Kaufmann SH. Ten challenges for TB biomarkers. *Tuberculosis (Edinb)* 2012; **92** Suppl 1: S17–S20.
37 Berry MP, Graham CM, McNab FW *et al*. An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis. *Nature* 2010; **466**: 973–977.
38 Menzies D, Al Jahdali H, Al Otaibi B. Recent developments in treatment of latent tuberculosis infection. *Indian J Med Res* 2011; **133**: 257–266.
39 Ehlers S. Lazy, dynamic or minimally recrudescent? On the elusive nature and location of the mycobacterium responsible for latent tuberculosis. *Infection* 2009; **37**: 87–95.
40 Trunz BB, Fine P, Dye C. Effect of BCG vaccination on childhood tuberculous meningitis and miliary tuberculosis worldwide: a meta-analysis and assessment of cost-effectiveness. *Lancet* 2006; **367**: 1173–1180.
41 Randhawa AK, Shey MS, Keyser A *et al*. Association of human TLR1 and TLR6 deficiency with altered immune responses to BCG vaccination in South African infants. *PLoS Pathog* 2011; **7**: e1002174.
42 Ritz N, Hanekom WA, Robins-Browne R, Britton WJ, Curtis N. Influence of BCG vaccine strain on the immune response and protection against tuberculosis. *FEMS Microbiol Rev* 2008; **32**: 821–841.
43 Brennan MJ, Clagett B, Fitzgerald H *et al*. Preclinical evidence for implementing a prime-boost vaccine strategy for tuberculosis. *Vaccine* 2012; **30**: 2811–2823.
44 *Critical path to TB drug regimens*. 2012. Available at http://cptrinitiative.org/ (accessed 1 June 2012).
45 Blumberg HM, Burman WJ, Chaisson RE *et al*. American Thoracic Society/Centers for Disease Control and Prevention/Infectious Diseases Society of America: treatment of tuberculosis. *Am J Respir Crit Care Med* 2003; **167**: 603–662.
46 World Health Organization. *Guidelines for programmatic management of drug-resistant tuberculosis*. Geneva: WHO Press, 2011. Available at http://whqlibdoc.who.int/publications/2011/9789241501583_eng.pdf. (accessed 14 February 2012).
47 Shah NS, Wright A, Bai GH *et al*. Worldwide emergence of extensively drug-resistant tuberculosis. *Emerging infectious diseases* 2007; **13**: 380–387.
48 McDermott W. Microbial persistence. *Yale J Biol Med* 1958; **30**: 257–291.
49 Fauvart M, de Groote VN, Michiels J. Role of persister cells in chronic infections: clinical relevance and perspectives on anti-persister therapies. *J Med Microbiol* 2011; **60**(Pt 6): 699–709.
50 Zhang Y, Yew WW, Barer MR. Targeting persisters for tuberculosis control. *Antimicrob Agents Chemother* 2012; **56**: 2223–2230.
51 Mitchison DA. The search for new sterilizing anti-tuberculosis drugs. *Front Biosci* 2004; **9**: 1059–1072.
52 Yasinskaya Y, Sacks L. Models and approaches for anti-TB drug testing. *Expert Rev Anti Infect Ther* 2011; **9**: 823–831.
53 Ma Z, Lienhardt C, McIlleron H, Nunn AJ, Wang X. Global tuberculosis drug development pipeline: the need and the reality. *Lancet* 2010; **375**: 2100–2109.
54 Miyata T, Cheigh CI, Casali N *et al*. An adjunctive therapeutic vaccine against reactivation and post-treatment relapse tuberculosis. *Vaccine* 2012; **30**: 459–465.
55 Van Deun A, Salim MA, Das AP, Bastian I, Portaels F. Results of a standardised regimen for multidrug-resistant tuberculosis in Bangladesh. *Int J Tuberc Lung Dis* 2004; **8**: 560–567.
56 National Institute of Allergy and Infectious Diseases. *Restructuring the NIAID Clinical Trials Networks*. Bethesda: National Institute of Allergy and Infectious Diseases; 2012. Available at http://www.niaid.nih.gov/labsandresources/restructuring (accessed 14 February 2012).
57 National Institute of Allergy and Infectious Diseases. *Resources for researchers*. Bethesda: National Institute of Allergy and Infectious Diseases; 2012. Available at http://www.niaid.nih.gov/labsandresources/resources (accessed 1 June 2012).
58 Nuermberger EL, Yoshimatsu T, Tyagi S, Bishai WR, Grosset JH. Paucibacillary tuberculosis in mice after prior aerosol immunization with Mycobacterium bovis BCG. *Infect Immun* 2004; **72**: 1065–1071.

59 McCune RM, Feldmann FM, Lambert HP, McDermott W. Microbial persistence. I. The capacity of tubercle bacilli to survive sterilization in mouse tissues. *J Exp Med* 1966; **123**: 445–468.
60 McCune RM, Feldmann FM, McDermott W. Microbial persistence. II. Characteristics of the sterile state of tubercle bacilli. *J Exp Med* 1966; **123**: 469–486.