Ref: Ro J Infect Dis. 2017;XX(1)
DOI: 10.37897/RJID.2017.1.1

Tuberculosis vaccines: current state and future directions

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ABSTRACT

Mycobacterium tuberculosis (Mtb) infects nearly 2 billion people worldwide, with around 9 million new cases and 1.5 million deaths from tuberculosis (TB) each year. Human immunodeficiency virus (HIV) is the only other infectious disease responsible for more deaths each year than *Mtb*. A strategy that combines faster methods of diagnosis and more efficient drug therapy and prevention (including new vaccines) is the only way to decrease TB morbidity and mortality. So far, vaccine Calmette-Guerin (BCG) is the only vaccine available against TB. Despite its proven effectiveness on reducing the incidence of meningitis and disseminated TB in children, he has a limited action against pulmonary forms in adolescents and adults and its use is unsafe in immunocompromised patients. The development of new vaccines has increased in the last 20 years, with more than 10 candidates in various stages of clinical trials. It is necessary to initiate new research directions, both to improve existing vaccines and to discover new ones.

Keywords: Tuberculosis, mycobacteria, prophylactic, vaccine, BCG, clinical trials

INTRODUCTION

In March 1993, the World Health Organization (WHO) declared TB, a disease caused by *Mtb*, a global public health emergency. Since 1993 there were more than 1.5 million deaths from TB every year. Almost one billion people diagnosed with TB have died in the last century and TB is the leading cause of mortality in HIV-infected patients. It is estimated that one third of the world's population is infected and 5-10% of them will develop active TB; the risk is considerably higher in the presence of predisposing factors, especially HIV, and other risk factors such as diabetes, renal failure, immunosuppressive drugs, smoking and other substances addiction (1).

In 2015, WHO reported 10.4 million new cases (incidence), of which 5.9 million (56%) were male, 3.5 million (34%) women and one million (10%) children. The incidence of new cases of HIV-infected patients in the same period was 1.2 million (11%) of all TB cases. There were a total of 1.4 million deaths from TB in 2015 and an additional 0.4 million deaths from TB in HIV-infected patients. Although the number of TB deaths fell by 22% during 2000 to 2015, TB remains one of the top 10 causes of death worldwide (2).

In our time TB raises two major issues: the emergence of chemoresistance, MDR-TB (chemoresistance in Isoniazid plus Rifampicin) and XDR-TB (extended chemoresistance), and the increased frequency of comorbidities. In 2015 there were reported 480,000 new cases of MDR-TB and a number of 100,000 patients with chemoresistance to Rifampicin. The latter is treated as an all MDR-TB, it is shown that 80% of patients with Rifampicin resistance have also resistance to Isoniazid (2).

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Article History:
Received: 12 February 2017
Accepted: 1 March 2017

Under these circumstances is an urgent need for new diagnostic methods and therapeutic and prophylactic diversification options. Current methods of TB prevention are: treatment of latent infection (LTBI), with special attention to children under 5 years who are contacts of bacteriogically confirmed pulmonary TB cases and for patients infected with HIV; prevention of transmission through rigorous control of *Mtb* infection; BCG vaccination of children according to each country's National Programmers (3).

THE IMMUNE RESPONSE TO THE *M. TUBERCULOSIS* INFECTION

The genesis of the pathological reactions in TB is inextricably linked with the response of the host to the invading tubercle bacillus. The events and outcomes that result from human exposure to *Mtb* are two phases: the acquisition of infection and the subsequent development of TB. In most individuals infected with *Mtb,* the host response restricts the growth of the pathogen and the disease gives up. TB may develop as direct progression from infection to disease (3% to 10% probability within one year of infection) or from late progression many years after infection (up to 5% probability for the life time of an infected person after the first year of infection).

In the case of *Mtb* infection the immune response initiated upon exposure follows a complex pathogenesis route. The main route of transmission for *Mtb* is via aerosolization of sputum liquid droplets containing bacilli that are then breathed in by an individual. After inhalation, organisms are carried into the deep lung where it is phagocytosed by antigen presenting cells, such as macrophages or dendritic cells, which then travel to the draining lymph nodes and initiate an immune response *Mtb*-specific T cells mediated.

CD4+T cells are the hallmark of the immune response to *Mtb* infection and, together with CD8+T cells play significant roles in the various stages of infection and disease. Then the T cells migrate to the lungs and are required for the formation of the TB specific granuloma around the site of infection. In an immunocompetent individual, the host defence mechanisms (the macrophages immune phagocytosis) prevent active disease. *Interferon (IFN)-γ and tumor necrosis factor (TNF)-α* are essential cytokines for the induction of immunity to infection. TNF-α contributes by activating microbicidal activities of macrophages and by modulating death of infected cells. IFN-γ is believed to contribute activating the microbicidal activities of macrophages, including autophagy, and by modulating inflammation at the site of infection. The principal cellular sources of IFN-γ are lymphocytes, including T cells, as well as adaptive CD4+ and CD8+T cells. *Interleukin-12 (IL-12)* is another essential cytokine for immunity to TB. The best-characterized role of IL-12 is in directing differentiation of CD4-T cells (Th1 cells) that secrete IFN-γ and contribute to control of TB.

Our understanding of the network of immune cells and cytokines required by the host to contain the infection, generate granulomas, and limit the extent of tissue involvement is increasing and will provide a better understanding of the requirements for generating vaccine mediated immunity (4).

The basis of a functional vaccine is dependent on the immune system's ability to "remember" an encounter with a foreign pathogen. Typically, immunological memory is established upon the first encounter with a pathogen by the creation of memory T cells. These memory T cells are specific to a particular antigen and will reside in tissues and lymph nodes until they recognize their specific pathogen and become activated. Upon activation by a second exposure, the memory T cells will quickly and efficiently initiate a response to eliminate the pathogen and prevent the disease. The creation of memory T cells and life-long immunity can be obtained either by primary exposure to the pathogen followed by disease and eventual recovery, or by being given a vaccine.

Vaccines act to prime the immune system by exposing a person to a non-lethal, milder version of the pathogen's antigens so that those memory T cells can be created without causing disease in the individual. Ideally, a vaccine is made of pathogen-derived components that are critical for induction of protective immunity and, consequently, are often composed of either an attenuated (non-virulent) or killed version of the bacteria, inactivated bacterial toxins, or subunits of the pathogen. The vaccines will have antigens similar to the virulent version of the pathogen but would lack factors necessary for disease (5,6).

Therefore, if a vaccinated person is exposed in secondary time to this particular set of antigens, the immune system will quickly eliminate the pathogen and prevent the disease. The immune system is required to respond specifically to both the vaccination as well as to pathogen-induced changes.

THE CURENT BCG VACCINE

By culturing a *M. bovis* strain isolated from a cow by Nocard in 1904 for a period of 13 years, and a total of 231 passages, on potato slices cooked in beef bile supplemented with glycerol, physician Calmette and Guérin, a veterinarian, at the Institut Pasteur in Lille, France, created a live, attenuated vaccine, bacillus Calmette-Guérin or BCG (7). The first human vaccination by Weil-Halle in Paris in 1921 was given to an infant by the oral route with several successive doses and the first mass immunization campaign of tuberculin skin test (TST) – negative individuals began in Poland in 1948 (8). Additional methods of administration employed subsequently include intradermal and percutaneous way through multiple stings or scarification. Since 1974, BCG vaccination of infants has been included in the WHO Expanded Programme on Immunization resulting in more than three billion cumulative vaccinations worldwide and approximately 100 million vaccinations per year (5).

Experimental studies indicate that the mechanism of protection by BCG vaccination consists in reduction of the hematogenous spread of bacilli from the site of primary infection mediated by memory T lymphocytes induced by the first exposure to BCG. There is no evidence that BCG reduces the risk of becoming infected with TB bacilli, but it prevents forms of TB depending on hematogenous spread of the bacillus; this category includes TB meningitis and miliary TB, which are associated with high mortality in children. This inhibition of the hematogenous spread of bacilli reduces the risk of direct progression from infection to disease and the disease due to endogenous reactivation. Because there is reduction in risk of immediate progression from infection to disease, but not of infection, there is a difference in the protective effect of BCG, depending on the TB pathogenesis route and the rapport infection-disease (8,9).

In their early studies on BCG vaccination, Calmette and Guérin already observed that BCG vaccination not only protected infants against death due to TB, but also reduced general mortality in infants who did not suffer from TB disease. This topic was largely ignored for decades. More recently, epidemiologic evidence has been presented from several low-income countries that BCG vaccination is correlated with reduced general infant mortality (10).

BCG vaccination should be provided as part of national childhood immunization programmes according to a country's TB epidemiology. In 2015, 163 countries reported providing BCG vaccination as a standard part of these programmes; 102 reported coverage of above 90%. Currently, WHO recommends that in countries with a high TB burden, a single dose of the BCG vaccine should be provided to all infants as soon as possible after birth, as part of childhood immunization programmes (2).

There is a clear need for a vaccine that is more effective than BCG, especially to reduce the risk of infection with *Mtb* and the risk of progression

from infection to active TB disease in adults. Although there are more than 10 candidates in the TB vaccine pipeline, a new TB vaccine is not expected in the near future (Table 1).

SPECIFIC DIFFICULTIES ASSOCIATED WITH *M. TUBERCULOSIS* VACCINE DEVELOPMENT

The complications involved with studying *Mtb* in a laboratory are numerous and range from the practical to the scientific. Due to the complex nature of the pathogen, *Mtb* presents some very unique challenges for the researcher studying it (6).

Biosafety considerations

When a researcher decides to investigate the development of a vaccine against *Mtb* one of the very first considerations that must be taken into account is the safety of those working with the pathogen. In the research laboratory setting, *Mtb* is considered a biosafety level 3 (BSL3) pathogen; a BSL3 pathogen is defined by the WHO as one that poses a high risk for an individual but alow risk for the community and can cause serious harm to an infected human. The various safety measures that must be put in place include laboratory practices, facility construction (separate rooms with negative pressure), security and safety equipment.

Slow growth of the pathogen

Mtb is a germ with slow multiplication, its doubling time being about 24 hours. For this reason, when researchers plan an experiment with infected animals, they should consider passing a sufficient duration of time between the initiation of the experiment and the study completion. Thus, if a vaccinated mice are administered a low dose of aerosol infected with virulent *Mtb*, it may take 3-4 weeks before granulomatous lesions in the lung are visible. In immunological research, mice are sacrificed in 30 to 120 days after infection, in order to highlight the long and short term immunity and whether the vaccine had any effect on reducing the number of mycobacteria.

Disease stages

In humans, infection with *Mtb* can be divided into four stages: infection, latency, active disease, and transmission (11). At each of these stages, the proteins expressed by the organism are thought to vary significantly and therefore protection in one stage may not protect in all.

After primary infection, in an immunologically healthy individual, an infection can progress to a LTBI. A person with a LTBI will not have active disease, but will have developed memory cells against *Mtb* antigens. When *Mtb* is in a latent stage it is slow growing or non-replicative and most likely expresses a different set of antigens than during the primary infection stage of the life cycle. When a person has a LTBI, their immune system is effectively containing the mycobacteria and they do not get sick. It is interesting to note that individuals that are infected with *Mtb* and progress on to latency are not protected from re-infection.

Ideally, the best vaccine would be one that provides protection from all stages of infection, i.e. a vaccine must be effective against primary infection, latency, endogenous reactivation and reinfection.

Vaccination in immunocompromised individuals

TB is the number one killer of HIV infected individuals.The resurgence of *Mtb* infections in the 1990s has largely been attributed to the advent of the HIV epidemic. An infection with HIV leads to a reduction in CD4+T cells and this is believed to be the cause of greater susceptibility to *Mtb* by most individuals. HIV infection may also disrupt the function of macrophages and *Mtb* specific T-cells that prevents the killing of the pathogen. The immune system of an HIV infected individual is, therefore, not able to control

the *Mtb* infection, resulting in reactivation of the disease. It has also been found that HIV-infected infants do not produce a robust Th1 immune response when vaccinated with BCG. Additionally, HIV positive infants are at a significantly increased risk of dissemination of BCG after vaccination. Another group of immunocompromised individuals that must be considered are those with poor living conditions and poor nutrition, diabetes, vitamin D deficiency, renal insufficiency, immunosuppressive drugs, tobacco and other substance addiction.

Vaccination pre- or post-exposure to the pathogen

In the case of TB, it is important to consider if the vaccine is aimed at pre- or post-exposure to the pathogen. As mentioned in the previous section, a person's immune system will respond differently to a vaccine if they have already been exposed to the pathogen. Since the immune system will have already been primed to respond to particular *Mtb* antigens that are not capable of protection, it is necessary to find a new method of immune activation. Because of the high prevalence of *Mtb* in endemic countries, it is likely that in order to eradicate TB, a vaccine will need to be effective post-exposure.

Laboratory strains *versus* clinical isolates

Mycobacteria from clinical isolates are often quite different than the strains commonly used in laboratory practice. Laboratories, by necessity for the repeatability of their experiments, must maintain an unchanging common lab strain. There are currently several strain types used in laboratories through out the world (H37Rv, HN878, Erdman, CDC1551 etc.) that vary in their virulence and antigenic composition. Unfortunately, however, these strains can be significantly different than the mycobacteria isolated from infected patients. The mycobacteria found "in the wild" is going to be constantly changing and mutating depending on various selection factors. These selection factors can include incomplete drug treatments or the strength of a non-drug treated person's immune response to the pathogen. Additionally, there is a natural mutation rate for the genome of all organisms independent of selection factors due to DNA replication errors or un-repaired DNA damage.The selection factors and mutational changes observed in clinical isolates are most apparent with the emergence of multi, extreme, and totally drug resistant organisms. Although it is hoped that there are enough similarities between laboratory strains and those isolated from patients, and so vaccination against some provide protection for all forms of *Mtb*, it should be kept in mind that constant mutation of "wild" strains exists, which should be considered.

Intrinsic properties of *Mtb* which make it difficult to immunize against

Mtb is intracellular, which means that the immune system must be able to recognize and then eliminate infected host cells without causing excessive damage to the host. *Mtb* has established several methods by which it can evade recognition by the host immune system. Most of these mechanisms revolve around ways of keeping the pathogen in a quiescent state within the macrophage. This block in phagocytosis also prevents proper presentation of *Mtb* antigens. Presentation of antigens is essential for recognition that an antigen is foreign and needs to be eliminated, as well as for the creation of immune memory. *Mtb* also evades removal by the ability to regulate both the necrotic and apoptotic death of host cells. All of the elimination avoidance methods developed by *Mtb* serve to maintain the pathogen within the macrophages where it can live for decades as a latent infection. Therefore, when designing a vaccine, it is necessary to activate the immune system in such a way that the host can overcome these evasion methods and recognize that the pathogen infected cells should be destroyed.

The future of *Mtb* vaccine development

Despite the slow decline of TB global incidence, the ominous prospect of increased incidence of MDR-TB emphasizes the need for new anti-TB vaccine that is more effective than BCG in preventing the disease. Ongoing clinical trials to develop new vaccines are shown in Table 1.

The structure of these vaccines include recombinant BCG-derived cell (rBCG), viral vectors, combinations of protein and adjuvant and mycobacterial extracts. The goal of these vaccines is to prevent infection (pre-exposure administration) to prevent progression of infection to disease or reactivation of LTBI (post-exposure administration), or immunotherapeutic in association with specific drugs (Table 1).

TB vaccines which are currently in different stages of clinical studies reflect several immunization strategies:

1) *A priming vaccine* to replace BCG with either live rBCG or genetically attenuated *Mtb* vaccines that confer greater safety and protective efficacy, to be given early in life and *before exposure to Mtb (13).* Priming vaccines are intended for use in newborns or young infants, i.e. at a time-point when the individual's immune system has not yet been exposed to natural infection with *Mtb* or other mycobacteria. BCG is given during the first weeks of life, whereas the viable vaccine candidates are supposed to replace BCG or considered as boosters of priming with BCG.

2) Another kind of vaccine *boosts the antimycobacterial immune response* either early or later in life *when LTBI is potentially installed*. Booster TB vaccines are vaccines that ideally can be given together with other childhood vaccines during the first year of life, but also at almost any timepoint, to schoolchildren, adolescents or adults, when the BCG's protection begins to dissipate. Vaccines of this type use either viral-vectored, adjuvanted subunit vaccines or heat-inactivated whole cell vaccines and require a different set of antigens (14). Also, the fact that later in life TB can arise both from endogenous reactivation and from exogenous reinfection may need to be reflected in the antigenic composition of a potent booster TB vaccine.

3) *A therapeutic vaccine against active TB*. Therapeutic vaccines target patients with active TB in adjunct to (and to shorten) chemotherapy or patients suffering from MDR-TB or XDR-TB. A candidate of this type is a killed *Mycobacterium vaccae* preparation that was used to treat patients with HIV coinfection suffering from miliary TB, with ambiguous outcome. RUTI, a semi

Table 1. *Overview of tuberculosis vaccines in clinical trials (2,3,11,12)*

Vaccine name	Target	Type	Phase	Developer
Ad5 Ag85A	Prime-boost	Viral vector	I	McMaster University, CanSino
TB/Flu-04L	Prime-boost	Viral vector	I	Research Ins tute for Biological Safety Problems
Crucell Ad35 + MVA85A	Prime-boost	Viral vector	I	Crucell, Oxford University, Aeras
ChAdO x 1.85A	Prime-boost	Viral vector	I	Oxford University
MTBVAC	Prime	Mycobacterial	I	University of Zaragoza, Biofabri, TuBerculosis Vaccine Ini a ve (TBVI)
MVA85A (aerosol)	Prime-boost	Viral vector	I	Oxford University
MVA85A-IMX313	Prime-boost	Viral vector	I	Oxford University, Imaxio
DAR-901	Prime-boost	Mycobacterial	IIa	Dartmouth University, Aeras
ID93 + GLA-SE	Prime-boost	Protein/adjuvant	IIa	Infec ous Disease Research Ins tute, Aeras, Aeras
RUTI	Imunotherapeu c	Mycobacterial	IIa	Archivel Farma, S.L
Hybrid 1 + IC31	Prime-boost	Protein/adjuvant	IIa	SSI, Valneva
Hybrid 4/Aeras-404 + IC31	Prime-boost	Protein/adjuvant	IIa	SSI, Sanofi Pasteur, Valneva, Aeras
Hybrid 56/Aeras-456 + IC31	Prime-boost	Protein/adjuvant	IIa	SSI, Valneva, Aeras
M72 + AS01E	Prime-boost	Protein/adjuvant	IIb	GlaxoSmithKline, Aeras
VPM 1002	Prime	Mycobacterial	IIb	Serum Ins tute of India, Vakzine Projekt Management, TBVI, Max Planck Ins tute for Infec on Biology
M. Vaccae	Imunotherapeu c	Mycobacterial	III	Anhui ZhifeiLongcon, China

purified preparation of killed *Mtb*, has completed phase II assessment in HIV-infected and HIV-uninfected individuals with LTBI.

Common to these strategies is a focus on achieving cell-mediated immunity by inducing Th1 cytokines (e.g., IFN-γ, TNF-α, and IL-2) produced by either CD-4 or CD-8 T cells. These cytokines activate other cells capable of inhibiting the growth of *Mtb* (11).

CONCLUSIONS

The BCG vaccine, despite its wide use, does not provide the needed protection. Effective vaccines will require a better understanding of the protective immune response to *Mtb* and of the pathogenesis of the disease. The availability of whole genomic sequences of thousands of clinical isolates of *Mtb* and the recent evidence of the importance of the coevolution of the host and organisms could assist the design of different vaccination approaches. We are still far from understanding why the natural immune system (which prevents 90% of persons infected from developing active TB) fails in 10% of those infected and why the adaptive immune response in TB does not protect us from repeated infections (15,16).

Although important scientific advances have been made recently, major gaps in understanding the basic biology of the organism and the human response to infection with *Mtb* remain. The rapidly emerging new challenges to the care and control of TB, such as HIV, MDR-TB, and more recently XDR-TB, may outstrip the advances and threaten our ability to control the disease.

Attributes of an ideal TB vaccine include safety and efficacy in neonates, infants, children and adults (including HIV infection); effectiveness against all forms of TB including pulmonary and MDR-TB; logistically practical (timing of vaccination and non-interference with other childhood immunizations); a formulation that can be feasibly manufactured on a mass scale and stored and administered under low-technology conditions.

Ongoing TB vaccine clinical trials are evaluating hypotheses for the variable efficacy of BCG; alterations to the methodology of BCG administration and novel candidate vaccines designed either to replace or enhance BCG (17).

Conflict of interest: none declared
Financial support: none declared

REFERENCES

1. **Evans T.G., Schrager L., Thole J.** Status of vaccine research and development of vaccines for tuberculosis. *Vaccine* 2016; 34: 2911-2914; https://www.ncbi.nlm.nih.gov/pubmed/26973073
2. **World Health Organization.** Global tuberculosis report 2016; http://www.who.int/tb/publications/global_report/en/
3. **Andersen P., Kaufmann S.H.E.** Novel Vaccination Strategies against Tuberculosis. *Cold Spring Harb Perspect Med* 2014; 4. doi: 10.1101/cshperspect.a018523
4. **Barker L.F., Hussey G.** The Immunological Basis for Immunization Series. Module 5: Tuberculosis. *World Health Organization* 2011; http://apps.who.int/iris/bitstream/10665/44733/1/9789241502412_eng.pdf
5. **Ottenhoff T.H.M., Kaufmann S.H.E.** Vaccines against Tuberculosis: Where Are We and Where Do We Need to Go? *PLoS Pathog* 2012; 8(5): e1002607, doi:10.1371/journal.ppat.1002607
6. **MacDonald E.M., Izzo A.A.** Tuberculosis Vaccine Development — Its History and Future Directions. *InTech* 2015. http://www.intechopen.com/books/tuberculosis-expanding-knowledge/tuberculosis-vaccine-development-its-history-and-future-directions
7. **Montagnani C., Chiappini E., Galli L., de Martino M.** Vaccine against tuberculosis: what's new? *BMC Infectious Diseases* 2014; 14 (Suppl 1): https://bmcinfectdis.biomedcentral.com/articles/10.1186/1471-2334-14-S1-S2
8. **Rowland L., McShane H.** Tuberculosis vaccines in clinical trials. Expert Rev Vaccines. 2011; 10 (5): 645-658. doi:10.1586/erv.11.28.
9. **Graves A.J., Hokey D.A.** Tuberculosis Vaccines: Review of Current Development Trends and Future Challenges. *J Bioterr Biodef* 2011; S1:009. http://dx.doi.org/10.4172/2157-2526.S1-009
10. **McShane H.** Tuberculosis vaccines: beyond bacille Calmette-Guérin. *Phil.Trans. R. Soc.* 2011; 366: 2782-2789. doi:10.1098/rstb.2011.0097
11. **Frick M.** The TB Vaccines Pipeline: Where are we going, where have we been? *Pipeline Report* 2013. http://www.stoptb.org/wg/mdrtb/resources/TAG/TBVaccines Pipeline.pdf
12. **Frick M.** The Tuberculosis Vaccines Pipeline: A New Path to the Same Destination? *Pipeline Report* 2016. http://www.pipelinereport.org/2015/tb-vaccines
13. **da Costa C., Walker B., Bonavia A.** Tuberculosis Vaccines – state of the art, and novel approaches to vaccine development. *Interna-*

tional Journal of Infectious Diseases 2015; 32: 5-12. https://www.ncbi.nlm.nih.gov/pubmed/25809749

14. **Tameris M.D., Hatherill M., Landry B.S., Scriba T.J., Snowden M.A., Lockhart S., Shea J.E., McClain J.B., Hussey G.D., Hanekom W.A., Mahomed H., McShane H., and the MVA85A 020 Trial Study Team.** Safety and efficacy of MVA85A, a new tuberculosis vaccine, in infants previously vaccinated with BCG: a randomised, placebo-controlled phase 2b trial. *Lancet* 2013; 381: 1021-28. https://www.ncbi.nlm.nih.gov/pubmed/23391465
15. **Hawn R.T., Day T.A., Scriba T.J., Hatherill M., Hanekom W.A., Evans T.G., Churchyard G.J., Kublin J.G., Bekker L.G., Self S.G.** Tuberculosis Vaccines and Prevention of Infection. *Microbiology and Molecular Biology Reviews* 2014; 78 (4) 650-671. doi:10.1128/MMBR.00021-14
16. **Karp C.L., Wilson C.B., Stuart L.M.** Tuberculosis vaccines: barriers and prospects on the quest for a transformative tool. *Immunological Reviews* 2015; 264: 363-381; https://www.ncbi.nlm.nih.gov/pubmed/25703572
17. **Barker L.F., Leadman A.E., Claget B.** The Challenges of Developing New Tuberculosis Vaccines. *Health Affairs* 30, 2011; 6: 1073-1079. doi: 10.1377/hlthaff.2011.0303