Introduction

In this introduction, I briefly review the natural history and pathogenesis of Tuberculosis (TB), with a focus on paediatric TB. This is followed by a summary of the global disease patterns and trends. Accordingly, I discuss the need for a new TB vaccine and the factors affecting low efficacy of the current extant vaccine. Given that there are potentially different vaccination strategies and targets, I review the selection of the target population, diagnostic considerations and practical elements for TB vaccine sites. The objectives of this thesis are to evaluate infants for suitability as a trial population, review diagnostic considerations which include evaluating the chest radiograph and the role of non-tuberculous mycobacteria. Finally, I look into the lessons learnt from the development of a TB vaccine trial site. These objectives are explained in more detail at the end of the introduction.

Pathogenesis of Tuberculosis

The causative agent of Tuberculosis is *Mycobacterium tuberculosis* complex (MTBC), mainly transmitted from person to person by droplets. Rarely, oral transmission occurs via unpasteurized milk. Thereafter, the course of the infection and disease is highly variable and to date, largely unpredictable. This is surprising, since MTBC has co-existed with man for millennia. In some individuals, certain immune mechanisms clear the infection before there is evidence of sensitization of adaptive immunity and these individuals can be resistant to TB infection (1-3). An unknown proportion of exposed persons develop latent or persistent infection (LTBI), defined as positive Tuberculin Skin Test (TST) or Interferon Gamma Release Assays (IGRAs) in the absence of clinical signs and symptoms of disease. It is estimated that about 1.7 billion individuals presently fall in this category (4). Of these, about 10% among those who are HIV uninfected will develop active disease in their lifetime. In some other individuals, infection progresses to disease, but they remain asymptomatic and capable of transmission (5). A minority develop clinically apparent disease with evidence of progressive disease in body organs plus microbiological confirmation. The incubation period varies, but it is typically thought to range from about three months to two years, and after that disease is relatively infrequent (6).

Paediatric TB

The course of paediatric TB is distinctly different from adult disease, particularly among young infants. TB is transmitted to infants through household contacts but mostly by exposures in the community (7). Studies from pre-chemotherapeutic era have attempted to quantify the natural history of the disease in children. After an infectious contact, an unknown proportion, but presumably higher than adults with similar exposure progress to primary infection. Of these, about 30-40% will progress to pulmonary disease after an incubation period of 3-8 weeks and 10-20% will develop disseminated disease one to three months after primary infection and approximately 50% of immunocompetent children will not develop disease (8). Infants therefore have a more severe course of disease with a higher proportion developing primary infection and disease within shorter time frames.

Epidemiology

Tuberculosis is the world's deadliest infectious disease, measured by the numbers of people who die each year, surpassing HIV and malaria (9). About 1.3 million people died of the disease in 2017. Those HIV infected are disproportionately affected. At least 30% of deaths among HIV infected persons are attributable to TB. There were at least 10 million people who suffered from TB in 2017, enduring social and financial distress (10, 11).

Global differences in TB burden

To optimize control strategies, it is important to map the disease and its determinants. There is considerable asymmetry in the epidemic. Of 193 nations in the world, five (3%) are responsible for more than half of the global disease burden. They are Brazil, Russia, India, China and South Africa (BRICS), characterized by steady economic growth over the last 15 years, but low commensurate reductions in TB deaths and incidence. This is attributed to economic inequalities, with certain population segments in these nations living under squalid conditions, with poor access to health care. This maldistribution nullifies the value of economic growth in driving down per capita TB, as happened in some nations.

There is considerable diversity in the age, estimated incidence and affected populations across these nations. Brazil (63/100,000), Russia (60/100,000) and China (63/100,000) have low incidence per capita, while India (204/100,000) and South Africa (567/100,000) have high incidence (12). In Brazil and Russia, TB burden is driven by transmission hotspots among vulnerable groups such as homeless persons and prisoners (12). China's disease rates are highest among the elderly who were sub-optimally treated prior to universal coverage of Directly Observed Therapy Short-course (DOTS) and set up of the National TB Program, resulting in high rates of reactivation (13). In India, patients seek care in the private sector first and frequently transition to the public sector, making it difficult for the program to track patients or assure quality of care. South Africa has the highest TB/HIV co-infection rates, and occupational hazards (mining) which drive incidence (14).

TB trends

Globally, the decline of incidence by 1.5% per year, observed over the last decade, will not achieve a world with 'zero deaths, disease and suffering due to TB by 2050'as envisioned by the STOP TB Partnership (14). The most recent targets envision a 95% reduction in TB mortality and incidence compared to the baseline period of 2015 (14). The most cost-effective long term solution for any infectious disease epidemic is effective vaccination (15, 16). This has been observed in cervical cancer (17, 18), polio (19), small pox, pneumonia (20). The discovery, development and rapid uptake of new interventions including a new TB vaccine are essential to the realization of a world free of TB (14).

The need for a new TB vaccine: Bacille Calmette-Guérin (BCG)

The only licensed TB vaccine has been administered for about a century (21). Its efficacy and cost-effectiveness against severe and disseminated forms of TB has been demonstrated (22). Protection against pulmonary TB is highly variable, between 0 to 80% (23), wanes after ten to fifteen years (24,

25) and only in one study lasted fifty years (26). The lack of consistent, durable protective efficacy against the most transmissible form of disease threatens all control efforts. The reasons for this, as well as BCG's exact mechanisms and correlates of protection are poorly understood.

Factors linked to BCG (in)efficacy

Presently, the diversity of BCG strains, age at vaccination, exposure to environmental mycobacteria and the route of BCG administration are all thought to influence BCG efficacy.

BCG strain

After development of BCG from a cow isolate in 1921, sub-cultures in various laboratories over time led to differences in BCG genotype and phenotype (27). Studies in mice have shown the strains have varying capacity to evoke delayed type hypersensitivity, T-cell cytotoxicity and proliferation (28). Strain specific differences in immune response and reactogenicity have also been shown in humans (29). However, whether strains affect vaccine efficacy in humans has not been demonstrated in head to head comparisons in clinical trials. This could suggest that apparent variances in immunogenicity do not correlate with protection (30).

Age at vaccination and revaccination

BCG is a neonatal vaccine administered intradermal. Administration at birth is supported by data showing BCG efficacy is highest when administered to unexposed (TST negative individuals), and those with low sensitivity to Non Tuberculous Mycobacteria (NTMs) (30), both conditions are assumed to be present in the early neonatal period. Additionally, BCG has beneficial off-target effects. They include promoting survival among low birth weight newborns, reducing all-cause mortality and infections in the neonatal period (31, 32) and improving heterologous Th1 responses to Tetanus Toxoid and polioviruses (31).

There have been concerns that new-born immunity is underdeveloped and could contribute to vaccine failure. When vaccination was deferred to 5-10 weeks of age, higher cytokine and T-cell responses were observed relative to neonatal vaccination (33, 34). It is not clear whether delayed vaccination translates to protective efficacy.

Delaying initial BCG vaccination to children aged 7-14 years with unknown TST status had modest (25%) efficacy against PTB (35). Boosting is a strategy to counter waning immune protection conferred by previous vaccination. Hence, the observed rise of TB incidence at the inset of adolescence ends the 'golden age' of lowest TB incidence. This led to the trial of BCG revaccination in the pre-adolescent period. However, this showed no efficacy (36). The authors conjectured that high helminth prevalence (37) may have reduced BCG efficacy in this instance. Notably, the inefficacy was present across all case definitions of TB. However, in the absence of Mantoux testing in the trial, some infected individuals may have received the vaccine, contributing to its non-efficacy (38).

Immune interference by NTMs

Contrary to long held dogma, the sterility of the lungs in normal physiology has been shown to be false (39). The lungs, like the gut, has a diverse microbiome, sources of which include micro-aspirations from upper respiratory tract, or in inhaled air (40). NTM species have been detected in human oral cavity and upper respiratory tract microbiomes in healthy subjects (41). They are likely critical for immune fitness and resistance to TB (42). Further, it has been speculated that high prevalence of NTM sensitization accounts for poor BCG efficacy in the tropics. This is mediated through cross reactive immune responses by masking (inducing an immune response to which BCG cannot improve on) or blocking (induction of a cross-reactive immune response, leading to non-replication of BCG and hence its inefficacy) (43). As such, they could be responsible for unquantified heterogeneity in vaccine responses among individuals. The species, incidence in sputum and clinical relevance of NTMs among BCG vaccinated infants in most high burden countries is unknown and will be reviewed in this thesis.

Route of administration

A large trial among South African infants found no differences in efficacy between the conventional intradermal versus sub-cutaneous BCG administration (44). Intranasal administered BCG has been tested in mice to mimic natural infection, with better protection (45, 46). No similar human trials have been conducted.

Trial design, endpoints & sample sizes

The BCG replacement vaccine, VPM1002 (www.prime-vaccine.eu) and other promising vaccine candidates (47, 48) are likely to progress to Phase III trials. In order to efficiently conduct these studies, reliable incidence estimates and cohort characterization are needed for sample size calculations, to inform trial design, selection of diagnostic tools and choice of endpoints as well as the target population.

Selection of target population

Traditionally, infants have been the natural target for new TB vaccines, since most vaccines are administered to them. BCG also has variety of beneficial target and off target effects (49, 50), which has ethical implications for the control arm in future trials. To optimize the limited resources available for TB vaccine development, numerous modelling studies have been conducted to identify the most appropriate target population, with conflicting results (51-55). Below I briefly review the arguments for neonatal vaccines. Thereafter, I also look at modelling studies which attempt to predict impact or cost-effectiveness of vaccinating neonates versus adults/adolescents versus mass vaccinations.

Neonatal TB vaccines

Conventional approaches to vaccination have failed to eliminate TB. As such the utility of neonates as a target population, must be reviewed against morbidity and mortality data as well as the potential impact of pre-infection vaccines on the End TB strategy. I also review the feasibility of a pre-infection vaccine.

Childhood TB morbidity

Challenging diagnosis

Childhood TB has been neglected by health programs. Children are considered of less public health import in disease transmission, as they frequently have pauci-bacillary disease. In addition, making the diagnosis is challenging. Signs and symptoms can be non-specific and usually associated with confounding co-morbidities such as acute pneumonia, HIV and undernutrition. Further, diagnostic aids such as sputum culture or GeneXpert, IGRAs, TSTs, expert radiograph assessment are either absent in high burden settings or lack sensitivity/specificity (56). There are also problems with under-reporting of confirmed pediatric cases to National TB Programs (57). Moreover, the lack of age disaggregated reports masks the high susceptibility of young children.

Inaccurate TB incidence estimates

The dearth of accurate TB incidence data also hampers design of interventional studies. Cohort studies deploying comprehensive diagnostic and follow up methods are few, due to the large sample sizes required (58, 59). Recently modelling studies have attempted to estimate the burden. Paediatric TB apparently contributes substantial fraction of the total global TB burden. Specifically, young children under five years bear more than half of the total incidence burden in children (60). Since modelling studies have inherent limitations (60), and program data are unreliable, cohort studies that systematically determine infant TB incidence are needed.

Childhood TB mortality

Given the paucity of incident data, non-specificity of signs and symptoms, co-existing lung pathologies and co-morbidities, it is possible an appreciable number of cases are missed. This has been confirmed by post-mortem studies (61, 62). What would be the case fatality rate of undiagnosed and therefore untreated disease? From the pre-chemotherapeutic era, at least 20% of children with untreated TB died from the disease within one year (63), with the majority of the deaths occurring among infants and toddlers. Not only is this is alarmingly high even compared to adults (64), it shows how fatal undiagnosed infant TB is and it is likely a top ten leading cause of early childhood mortality (65). Therefore, young children have a high unmet need for a new effective vaccine and constitute a top priority population.

Feasibility of a pre-infection vaccine

Neonates are presumed to be uninfected with TB. They are therefore targeted for pre-infection vaccines, which seek to prevent onset of TB infection and halt disease progression.

Simple versus complex pathogens: A question remains whether a pre-infection vaccine is even feasible? With simple pathogens, for example viruses, disease incidence is a function of infection. Hence, halving infection rates for example by vaccination, halves the disease incidence rates. This is not necessarily true for TB since MTBC has a complex pathogenic process, where infection often does not correlate with disease. There are latently infected people who don't progress to disease, but there

are also infected persons who also advance quickly to disease. Pre-infection vaccines would act on such susceptible persons who rapidly progress to disease for example infants (52).

Immunological precedence: There is some evidence BCG prevents infection in neonates, albeit from non-randomized studies (66). Recently, a trial of BCG vs H4IC31 vs placebo showed both vaccines incapable of preventing initial QFT conversion among QFT negative adolescents (48). Nevertheless, in natural infections, after MTBC exposure, it takes about six weeks to establish CD4 T cell responses, providing a window for vaccine induced immunity to combat MTBC evasion strategies, clearing the pathogen and preventing persistence of infection (67). Natural history studies show some individuals to be persistently TST/IGRA negative despite continued exposure (68). Hence, it appears that prevention of infection vaccines for neonates could have immunological precedence.

Modelling studies in selecting the target trial population

Pre-infection vaccines (Prevention of Infection/PoI)

Modelling studies show pre-infection vaccines targeting TB unexposed neonates would substantially reduce the burden of new infections. This effect would increase over time. On the other hand, post-exposure vaccines, targeting adults/adolescents would only minimally reduce the burden of new infections, and this effect would diminish over time (52).

Post-exposure vaccines (Prevention of Disease/PoD)

Modelling studies support greater and more rapid impact of adolescent/adult targeted prevention of disease vaccines in reducing the new number of cases of disease, over neonatal pre-exposure vaccines (51-53). Since infants do not transmit disease and it would be about 10-20 years before they are at increased risk of transmissible disease, a similar lag is expected before neonatal vaccination can impact the TB epidemic (54, 69). Further, economic evaluation models shows vaccinating adults to be remarkably cost effective (51). Recently released results of the phase IIb trial of M72/AS01E vs placebo in adults with LTBI showed 49.7% efficacy in preventing disease.

CONCLUSION

Modelling studies have value in conceptualizing multiple scenarios of vaccine coverage, efficacy, duration of protection and secular factors that affect disease epidemiology. They have inherent limitations which I describe below. Secondly, modelling studies are inconclusive on pre vs post-exposure vaccination. Some authors found neither pre nor post-exposure vaccines would reduce high incidence epidemics due to the complex pathogenic process of TB (52). A systematic review of 23 mathematical models exploring the potential impact of TB vaccines considered factors that would explain the inconclusiveness of pre *vs.* post-exposure vaccination (54).

Limitations of modelling:

a. External factors and geographical bias: Most models are based on an Asia like epidemic, driven by reactivation disease. Majority exclude Sub-Saharan Africa, where disease is driven by new infections (70, 71). If the rate of treatment of active disease would increase as envisioned in the present strategy (14), infections and thereby disease would decline, decreasing the relative impact of a pre-exposure vaccine in Asia like epidemics. Whereas a post-exposure vaccine would be found more useful in settings where reactivation disease drives the epidemic such as Asia (72).
b. The prevailing rates of LTBI: As few models report the LTBI rates used, it is difficult to assess comparative numbers of people most likely to benefit from post-exposure over pre-exposure vaccines (21).
c. HIV and Anti-Retroviral therapy status is largely ignored: It impacts longevity and probability of disease, vaccine efficacy (52, 54).
d. Availability of vaccination platforms and vaccine hesitance in adults is assumed in computing vaccine coverage. Vaccine hesitance is one of the top 10 global threats (73). There are few studies that have investigated the feasibility and acceptability of an adult TB vaccine. Developing a vaccination platform in most high burden countries will be costly and require complex logistical operations for health systems which are underfunded and burdened by providing care. This could undermine cost-effectiveness of an adult vaccine.

Diagnostic and endpoint considerations

Endpoints

In addition to identifying the target population, selection of endpoints also determine efficacy, as well as trial cost and duration. Non-microbiologically confirmed TB (Clinical TB) endpoints have been shown to undermine vaccine efficacy, given the vaccine is not designed to prevent non-TB respiratory ailments. The results of the M72/ASO1$_E$ vaccine trial confirmed this. Vaccine efficacy diminished proportional to the diagnostic distance from culture and molecular confirmation (47). However, when successful vaccine candidates move to efficacy trials, the relative rarity of incident TB necessitates sample sizes of tens of thousands of persons. To mitigate the risks of failure of a candidate after such a colossal investment, target vaccine profiles have been redefined to achieve lower threshold, 'proof of concept' end-points, namely: Prevention of Infection (initial or sustained IGRA conversion); Prevention of Disease (POD); Prevention of Recurrence (PoR) and therapeutic vaccines (67).

Endpoints: Prevention of infection endpoints.

Prevention of infection endpoints require significantly lower sample sizes (48, 67) and shorter durations of follow up as the incidence of latent infection is greater than disease in high burden countries. Hence efficacy in PoI acts as surrogate and potential proof of efficacy against disease and can be needed to proceed to much larger PoD efficacy studies.

Endpoints: Prevention of disease

Despite the attraction of PoI vaccines, PoD is really the most clinically meaningful end-point. Most latently infected persons have a low probability of progressing to disease, and further, prevention of initial or sustained QFT conversion, may not translate to PoD. Ultimately PoD trials are inevitable.

Chest radiograph (CXR)

Composite endpoints that include chest radiograph assessments have been used to define TB disease in trials. It is an invaluable adjunct of diagnosis. Nevertheless, it is fraught with risks. Most patients do not have classical radiological features (74), and the co-existence of multiple lung pathogens and co-morbidities among young children with TB confound the radiological diagnosis. For example, a comparison of radiological findings between severely under-nourished children with confirmed TB and those without, found no differences (75). Also a post-mortem study between the ante mortem radiographs of HIV infected and uninfected children found no difference in radiologic findings (76). Nevertheless, the specificity and reproducibility can be improved by using expert adjudication of radiographs.

Practical considerations: Site selection and development

Licensure trials for new PoI and PoD vaccines are likely to occur in high TB incidence countries. Most such settings lack the requisite clinical trial infrastructure and experience in conducting large studies. Infant mortality also tends to be high which can further undermine case ascertainment if there are numerous early deaths due to unrelated causes. Hence, large cohort studies deploying comprehensive diagnostic methods were needed to obtain incidence and mortality estimates.

Trial site description-Western Kenya

Outside of the BRICS countries, there are 25 other high burden countries, one of which is Kenya. The estimated prevalence of TB disease is 558/100,000 and about only half of those with the disease are treated (77).The estimated incidence is 319/100,000, with HIV co-infection rates of 29% (12). TB rates are highest among males and those aged between 25 and 30 years (77). The exact drivers of the epidemic have not been well characterized. The study site described in this thesis was set up following receipt of funding and co-funding from a consortium of partners to create capacity to conduct TB vaccine trials in 2007. A large complement of data and laboratory staff, nurses, pharmacists, doctors and clinical officers received training and practical experience in trial related procedures.

In 2009, a large cohort of neonates were enrolled to determine TB incidence (Infant Cohort Study). It was a landmark study that had not been done previously in the country nor has it been replicated since in Kenya. The cost investment of such studies is prohibitively high and therefore the lessons mined are to be used to optimize future vaccine trials.

AIMS OF THESIS

Overall, the aim of this thesis was to determine the suitability of infants as a trial population and inform diagnostic considerations for future TB vaccine trials. This included documenting the practical experience of site set up and trial implementation as well as the lessons for TB vaccine development. These questions are addressed in TB studies particularly among infants.

The specific objectives were:

To evaluate infants for suitability as a target population

- To assess TB incidence, post-neonatal mortality and cohort retention among infants.

Diagnostic considerations

- To evaluate the chest radiograph (CXR) for its suitability as an endpoint for infant trials where paucibacillary disease is most frequent.
- To determine the clinical relevance of non-tuberculous mycobacteria isolated in sputum, given their ubiquity and indistinguishable case presentation to TB disease.

Lessons from site development and for the TB vaccine pipeline

- To reflect on the lessons learnt in building a new TB vaccine site and review the TB vaccine development pipeline in the context of the Ebola virus outbreak.

OUTLINE OF THESIS

Chapter 2 describes the incidence of TB disease among infants, as a potential trial population. This chapter differentiates incident TB based on whether it is microbiologically confirmed or not and calculates the sensitivity of sample sizes for each case definition to determine the suitability of infants as a target population. We also examine retention, which also influences sample size. **Chapter 3.** High infant mortality is an important cohort characteristic of most high incidence countries, it undermines the ability to detect endpoints. In such settings, how much mortality can we anticipate in infant trials, and how much of it is due to background morbidity? This section determines the post-neonatal mortality in the study area, also the determinants and causes of mortality. **Chapter 4** examines the diagnostic utility of the chest radiograph for defining non-microbiologically confirmed TB endpoints. What is the inter-rater agreement of expert and non-expert readers in assessing radiographs for consistency with TB? The implications of including the expert readings of the chest radiograph on TB incidence, vaccine efficacy and sample size calculations will be reviewed. **Chapter 5** defines the species, clinical relevance, diagnostic difficulties and incidence of the ubiquitous non-tuberculous mycobacteria among infants with presumptive TB. **Chapter 6.** Phase IIb and III TB vaccine trials require large sample sizes. Therefore, multiple sites with the requisite disease burden, trial infrastructure and

expertise are needed in high incidence countries. This chapter reviews the challenges and opportunities of developing a TB vaccine site against the backdrop of the momentous breakthrough that was the demonstration of efficacy of the Ebola virus vaccine. In the discussion, I summarize our findings in relation to questions raised in this thesis, contextualize our results and provide future perspectives in light of the lessons learnt.

REFERENCES

1. Ma N, Zalwango S, Malone LL, Nsereko M, Wampande EM, Thiel BA, et al. Clinical and epidemiological characteristics of individuals resistant to M. tuberculosis infection in a longitudinal TB household contact study in Kampala, Uganda. BMC Infect Dis. 2014;14:352.
2. Simmons JD, Stein CM, Seshadri C, Campo M, Alter G, Fortune S, et al. Immunological mechanisms of human resistance to persistent Mycobacterium tuberculosis infection. Nat Rev Immunol. 2018;18(9):575-89.
3. Mave V, Chandrasekaran P, Chavan A, Shivakumar S, Danasekaran K, Paradkar M, et al. Infection free "resisters" among household contacts of adult pulmonary tuberculosis. PLoS One. 2019;14(7):e0218034.
4. Houben RM, Dodd PJ. The Global Burden of Latent Tuberculosis Infection: A Re-estimation Using Mathematical Modelling. PLoS Med. 2016;13(10):e1002152.
5. Mao TE, Okada K, Yamada N, Peou S, Ota M, Saint S, et al. Cross-sectional studies of tuberculosis prevalence in Cambodia between 2002 and 2011. Bull World Health Organ. 2014;92(8):573-81.
6. Behr MA, Edelstein PH, Ramakrishnan L. Revisiting the timetable of tuberculosis. BMJ. 2018;362:k2738.
7. Martinez L, Shen Y, Mupere E, Kizza A, Hill PC, Whalen CC. Transmission of Mycobacterium Tuberculosis in Households and the Community: A Systematic Review and Meta-Analysis. Am J Epidemiol. 2017;185(12):1327-39.
8. Marais BJ, Gie RP, Schaaf HS, Hesseling AC, Obihara CC, Starke JJ, et al. The natural history of childhood intra-thoracic tuberculosis: a critical review of literature from the pre-chemotherapy era. Int J Tuberc Lung Dis. 2004;8(4):392-402.
9. World Health Organization; Geneva. Global Health Estimates 2016: Disease burden by Cause, Age, Sex, by Country and by Region, 2000-2016. 2018.
10. Prasanna T, Jeyashree K, Chinnakali P, Bahurupi Y, Vasudevan K, Das M. Catastrophic costs of tuberculosis care: a mixed methods study from Puducherry, India. Glob Health Action. 2018;11(1):1477493.
11. World Health Organization (WHO) End TB Strategy. 2014.
12. World Health Organization (WHO); Geneva. Global tuberculosis report 2018.
13. Leung CC, Lam TH, Chan WM, Yew WW, Ho KS, Leung GM, et al. Diabetic control and risk of tuberculosis: a cohort study. Am J Epidemiol. 2008;167(12):1486-94.
14. World Health Organisation (WHO); The End TB Strategy 2017.
15. McShane H. Understanding BCG is the key to improving it. Clin Infect Dis. 2014;58(4):481-2.
16. Greenwood B. The contribution of vaccination to global health: past, present and future. Philos Trans R Soc Lond B Biol Sci. 2014;369(1645):20130433.
17. Vorno T, Lutsar K, Uuskula A, Padrik L, Raud T, Reile R, et al. Cost-effectiveness of HPV vaccination in the context of high cervical cancer incidence and low screening coverage. Vaccine. 2017;35(46):6329-35.
18. Ng SS, Hutubessy R, Chaiyakunapruk N. Systematic review of cost-effectiveness studies of human papillomavirus (HPV) vaccination: 9-Valent vaccine, gender-neutral and multiple age cohort vaccination. Vaccine. 2018;36(19):2529-44.
19. Geneva WHO. Polio vaccines: WHO position paper Weekly epidemiological record. 2016.
20. Chen C, Cervero Liceras F, Flasche S, Sidharta S, Yoong J, Sundaram N, et al. Effect and cost-effectiveness of pneumococcal conjugate vaccination: a global modelling analysis. Lancet Glob Health. 2019;7(1):e58-e67.
21. Dye C, Glaziou P, Floyd K, Raviglione M. Prospects for tuberculosis elimination. Annu Rev Public Health. 2013;34:271-86.

22. 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(9517):1173-80.
23. Barreto ML, Pereira SM, Ferreira AA. BCG vaccine: efficacy and indications for vaccination and revaccination. J Pediatr (Rio J). 2006;82(3 Suppl):S45-54.
24. Nguipdop-Djomo P, Heldal E, Rodrigues LC, Abubakar I, Mangtani P. Duration of BCG protection against tuberculosis and change in effectiveness with time since vaccination in Norway: a retrospective population-based cohort study. Lancet Infect Dis. 2016;16(2):219-26.
25. Abubakar I, Pimpin L, Ariti C, Beynon R, Mangtani P, Sterne JA, et al. Systematic review and meta-analysis of the current evidence on the duration of protection by Bacille Calmette-Guérin vaccination against tuberculosis. Health Technol Assess. 2013;17(37):1-372, v-vi.
26. Aronson NE, Santosham M, Comstock GW, Howard RS, Moulton LH, Rhoades ER, et al. Long-term efficacy of BCG vaccine in American Indians and Alaska Natives: A 60-year follow-up study. JAMA. 2004;291(17):2086-91.
27. Bottai D, Brosch R. The BCG Strain Pool: Diversity Matters. Mol Ther. 2016;24(2):201-3.
28. Lagranderie MR, Balazuc AM, Deriaud E, Leclerc CD, Gheorghiu M. Comparison of immune responses of mice immunized with five different Mycobacterium bovis BCG vaccine strains. Infect Immun. 1996;64(1):1-9.
29. Anderson EJ, Webb EL, Mawa PA, Kizza M, Lyadda N, Nampijja M, et al. The influence of BCG vaccine strain on mycobacteria-specific and non-specific immune responses in a prospective cohort of infants in Uganda. Vaccine. 2012;30(12):2083-9.
30. Mangtani P, Abubakar I, Ariti C, Beynon R, Pimpin L, Fine PE, et al. Protection by BCG vaccine against tuberculosis: a systematic review of randomized controlled trials. Clin Infect Dis. 2014;58(4):470-80.
31. Biering-Sørensen S1, Aaby P2, Lund N1,2, Monteiro I2, Jensen KJ1,3, Eriksen HB1,2, Schaltz-Buchholzer F2, Jørgensen ASP2, Rodrigues A2, Fisker AB1,2, Benn CS1,4. Early BCG-Denmark and Neonatal Mortality Among Infants Weighing <2500 g: A Randomized Controlled Trial. Clin Infect Dis. 2017.
32. Kjaergaard J. Bacille Calmette-Guérin vaccination at birth: Effects on early childhood infections, growth, and development. Dan Med J. 2016;63(11).
33. Hur YG, Gorak-Stolinska P, Lalor MK, Mvula H, Floyd S, Raynes J, et al. Factors affecting immunogenicity of BCG in infants, a study in Malawi, The Gambia and the UK. BMC Infect Dis. 2014;14:184.
34. Kagina BM, Abel B, Bowmaker M, Scriba TJ, Gelderbloem S, Smit E, et al. Delaying BCG vaccination from birth to 10 weeks of age may result in an enhanced memory CD4 T cell response. Vaccine. 2009;27(40):5488-95.
35. Pereira SM, Barreto ML, Pilger D, Cruz AA, Sant'Anna C, Hijjar MA, et al. Effectiveness and cost-effectiveness of first BCG vaccination against tuberculosis in school-age children without previous tuberculin test (BCG-REVAC trial): a cluster-randomised trial. Lancet Infect Dis. 2012;12(4):300-6.
36. Rodrigues LC, Pereira SM, Cunha SS, Genser B, Ichihara MY, de Brito SC, et al. Effect of BCG revaccination on incidence of tuberculosis in school-aged children in Brazil: the BCG-REVAC cluster-randomised trial. Lancet. 2005;366(9493):1290-5.
37. Prado M, Barreto ML, Strina A, Faria JA, Nobre AA, Jesus SR. [Prevalence and intensity of infection by intestinal parasites in school-aged children in the City of Salvador (Bahia, Brazil)]. Rev Soc Bras Med Trop. 2001;34(1):99-101.
38. World Health Organization (WHO); WHO statement on BCG revaccination for the prevention of tuberculosis. Bulletin of the World Health Organization. 1995;73(‍ 6)‍ : :805-6.
39. Ren L, Zhang R, Rao J, Xiao Y, Zhang Z, Yang B, et al. Transcriptionally Active Lung Microbiome and Its Association with Bacterial Biomass and Host Inflammatory Status. mSystems. 2018;3(5).

40. Dickson RP, Erb-Downward JR, Martinez FJ, Huffnagle GB. The Microbiome and the Respiratory Tract. Annu Rev Physiol. 2016;78:481-504.
41. Macovei L, McCafferty J, Chen T, Teles F, Hasturk H, Paster BJ, et al. The hidden 'mycobacteriome' of the human healthy oral cavity and upper respiratory tract. J Oral Microbiol. 2015;7:26094.
42. Gupta N, Kumar R, Agrawal B. New Players in Immunity to Tuberculosis: The Host Microbiome, Lung Epithelium, and Innate Immune Cells. Front Immunol. 2018;9:709.
43. Brandt L, Orme I. Prospects for new vaccines against tuberculosis. Biotechniques. 2002;33(5):1098, 100, 102.
44. Hawkridge A, Hatherill M, Little F, Goetz MA, Barker L, Mahomed H, et al. Efficacy of percutaneous versus intradermal BCG in the prevention of tuberculosis in South African infants: randomised trial. BMJ. 2008;337:a2052.
45. Bull NC, Stylianou E, Kaveh DA, Pinpathomrat N, Pasricha J, Harrington-Kandt R, et al. Enhanced protection conferred by mucosal BCG vaccination associates with presence of antigen-specific lung tissue-resident PD-1(+) KLRG1(-) CD4(+) T cells. Mucosal Immunol. 2019;12(2):555-64.
46. Giri PK, Sable SB, Verma I, Khuller GK. Comparative evaluation of intranasal and subcutaneous route of immunization for development of mucosal vaccine against experimental tuberculosis. FEMS Immunol Med Microbiol. 2005;45(1):87-93.
47. Van Der Meeren O, Hatherill M, Nduba V, Wilkinson RJ, Muyoyeta M, Van Brakel E, et al. Phase 2b Controlled Trial of M72/AS01E Vaccine to Prevent Tuberculosis. N Engl J Med. 2018;379(17):1621-34.
48. Nemes E, Geldenhuys H, Rozot V, Rutkowski KT, Ratangee F, Bilek N, et al. Prevention of M. tuberculosis Infection with H4:IC31 Vaccine or BCG Revaccination. N Engl J Med. 2018;379(2):138-49.
49. Nankabirwa V, Tumwine JK, Mugaba PM, Tylleskar T, Sommerfelt H, Group P-ES. Child survival and BCG vaccination: a community based prospective cohort study in Uganda. BMC Public Health. 2015;15:175.
50. Roth AE, Stensballe LG, Garly ML, Aaby P. Beneficial non-targeted effects of BCG--ethical implications for the coming introduction of new TB vaccines. Tuberculosis (Edinb). 2006;86(6):397-403.
51. Knight GM, Griffiths UK, Sumner T, Laurence YV, Gheorghe A, Vassall A, et al. Impact and cost-effectiveness of new tuberculosis vaccines in low- and middle-income countries. Proc Natl Acad Sci U S A. 2014;111(43):15520-5.
52. Ziv E, Daley CL, Blower S. Potential public health impact of new tuberculosis vaccines. Emerg Infect Dis. 2004;10(9):1529-35.
53. Lietman T, Blower SM. Potential impact of tuberculosis vaccines as epidemic control agents. Clin Infect Dis. 2000;30 Suppl 3:S316-22.
54. Harris RC, Sumner T, Knight GM, White RG. Systematic review of mathematical models exploring the epidemiological impact of future TB vaccines. Hum Vaccin Immunother. 2016;12(11):2813-32.
55. Abu-Raddad LJ, Sabatelli L, Achterberg JT, Sugimoto JD, Longini IM, Jr., Dye C, et al. Epidemiological benefits of more-effective tuberculosis vaccines, drugs, and diagnostics. Proc Natl Acad Sci U S A. 2009;106(33):13980-5.
56. Kaguthi G, Nduba V, Nyokabi J, Onchiri F, Gie R, Borgdorff M. Chest Radiographs for Pediatric TB Diagnosis: Interrater Agreement and Utility. Interdiscip Perspect Infect Dis. 2014;2014:291841.
57. du Preez K, Schaaf HS, Dunbar R, Swartz A, Bissell K, Enarson DA, et al. Incomplete registration and reporting of culture-confirmed childhood tuberculosis diagnosed in hospital. Public Health Action. 2011;1(1):19-24.
58. Moyo S, Verver S, Hawkridge A, Geiter L, Hatherill M, Workman L, et al. Tuberculosis case finding for vaccine trials in young children in high-incidence settings: a randomised trial. Int J Tuberc Lung Dis. 2012;16(2):185-91.

59. López-Varela E1 AO, Gondo K, García-Basteiro AL, Fraile O, Ira T, Ribó Aristizabal JL, Bulo H, Muñoz Gutierrez J, Aponte J, Macete E, Sacarlal J, Alonso PL. Incidence of Tuberculosis Among Young Children in Rural Mozambique. Pediatr Infect Dis J. 2015.
60. Dodd PJ, Gardiner E, Coghlan R, Seddon JA. Burden of childhood tuberculosis in 22 high-burden countries: a mathematical modelling study. Lancet Glob Health. 2014;2(8):e453-9.
61. Bates M, Shibemba A, Mudenda V, Chimoga C, Tembo J, Kabwe M, et al. Burden of respiratory tract infections at post mortem in Zambian children. BMC Med. 2016;14:99.
62. Chintu C, Mudenda V, Lucas S, Nunn A, Lishimpi K, Maswahu D, et al. Lung diseases at necropsy in African children dying from respiratory illnesses: a descriptive necropsy study. Lancet. 2002;360(9338):985-90.
63. Jenkins HE, Yuen CM, Rodriguez CA, Nathavitharana RR, McLaughlin MM, Donald P, et al. Mortality in children diagnosed with tuberculosis: a systematic review and meta-analysis. Lancet Infect Dis. 2017;17(3):285-95.
64. Tiemersma EW, van der Werf MJ, Borgdorff MW, Williams BG, Nagelkerke NJ. Natural history of tuberculosis: duration and fatality of untreated pulmonary tuberculosis in HIV negative patients: a systematic review. PLoS One. 2011;6(4):e17601.
65. Dodd PJ, Yuen CM, Sismanidis C, Seddon JA, Jenkins HE. The global burden of tuberculosis mortality in children: a mathematical modelling study. Lancet Glob Health. 2017;5(9):e898-e906.
66. Roy A, Eisenhut M, Harris RJ, Rodrigues LC, Sridhar S, Habermann S, et al. Effect of BCG vaccination against Mycobacterium tuberculosis infection in children: systematic review and meta-analysis. BMJ. 2014;349:g4643.
67. Hawn TR, Day TA, Scriba TJ, Hatherill M, Hanekom WA, Evans TG, et al. Tuberculosis vaccines and prevention of infection. Microbiol Mol Biol Rev. 2014;78(4):650-71.
68. Mahan CS, Zalwango S, Thiel BA, Malone LL, Chervenak KA, Baseke J, et al. Innate and adaptive immune responses during acute M. tuberculosis infection in adult household contacts in Kampala, Uganda. Am J Trop Med Hyg. 2012;86(4):690-7.
69. Young DB, Perkins MD, Duncan K, Barry CE, 3rd. Confronting the scientific obstacles to global control of tuberculosis. J Clin Invest. 2008;118(4):1255-65.
70. Middelkoop K, Bekker LG, Mathema B, Shashkina E, Kurepina N, Whitelaw A, et al. Molecular epidemiology of Mycobacterium tuberculosis in a South African community with high HIV prevalence. J Infect Dis. 2009;200(8):1207-11.
71. Mekonnen A, Merker M, Collins JM, Addise D, Aseffa A, Petros B, et al. Molecular epidemiology and drug resistance patterns of Mycobacterium tuberculosis complex isolates from university students and the local community in Eastern Ethiopia. PLoS One. 2018;13(9):e0198054.
72. Dye C, Williams BG. Eliminating human tuberculosis in the twenty-first century. J R Soc Interface. 2008;5(23):653-62.
73. Larson HJ. The state of vaccine confidence. Lancet. 2018;392(10161):2244-6.
74. Garcia-Basteiro AL, Lopez-Varela E, Augusto OJ, Gondo K, Munoz J, Sacarlal J, et al. Radiological findings in young children investigated for tuberculosis in Mozambique. PLoS One. 2015;10(5):e0127323.
75. Chisti MJ, Graham SM, Duke T, Ahmed T, Ashraf H, Faruque AS, et al. A prospective study of the prevalence of tuberculosis and bacteraemia in Bangladeshi children with severe malnutrition and pneumonia including an evaluation of Xpert MTB/RIF assay. PLoS One. 2014;9(4):e93776.
76. Rennert WP, Kilner D, Hale M, Stevens G, Stevens W, Crewe-Brown H. Tuberculosis in children dying with HIV-related lung disease: clinical-pathological correlations. Int J Tuberc Lung Dis. 2002;6(9):806-13.
77. Enos M, Sitienei J, Ong'ang'o J, Mungai B, Kamene M, Wambugu J, et al. Kenya tuberculosis prevalence survey 2016: Challenges and opportunities of ending TB in Kenya. PLoS One. 2018;13(12):e0209098.