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TB vaccine trials in Kenya

Preparation and implementation

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Publication date

2020

Document Version

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Citation for published version (APA):

Nduba, V. N. (2020). *TB vaccine trials in Kenya: Preparation and implementation*.

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CHAPTER 9

General Discussion

We sought to establish a platform for the evaluation of potential TB vaccines. We conducted epidemiological studies in infants and adolescents to both establish the research capacity to conduct TB vaccine trials and obtain TB incidence estimates to inform future TB vaccine trials. In our study population, we found a high incidence of TB infection and disease among infants and adolescents. The low patient diagnostic rate indicated poor performance of the routine TB control program and warrants further action to improve case detection, including targeting youth in and out of school and not only adults for active case finding to find undetected cases. The high annual risk of TB infection (ARTI) and high tuberculin skin test (TST) conversion rates during follow up indicated a high force of infection in this community and implementation of isoniazid preventive therapy in this population should be considered. Recent data from the phase II trial of the M72/AS01E TB vaccine has shown a higher efficacy (54%) than what the World Health Organization (WHO) defines as the needed minimum efficacy for a vaccine targeting prevention of disease (40%) for impacting the TB epidemic(1) (2) and may be appropriate for this community. As promising TB vaccine candidates move to phase III registration trials, the need for large sample sizes will require multiple sites in high burden and probably low resource settings creating the need to continue capacity building more trial sites, such as the one we built and described in this thesis.

In addition to data supporting TB vaccine development, we also collected relevant data for TB control. We found a high prevalence of tuberculosis disease among adolescents with the majority of cases found not having been previously diagnosed by the national TB control program(**Chapter 2**). The missed cases are consistent with a recently concluded national wide TB prevalence survey that found 40% of cases are missed and the estimated incidence rate from the survey was almost double the estimate reported by WHO(3). Further, additional tools, with better sensitivity like GeneXpert Ultra and LED microscopy should be deployed by the TB control program to increase detection of TB in adolescents since the majority were smear negative. Out of school youth were under enrolled and being out of school was a risk factor for prevalent tuberculosis. Targeting schools only for active case finding would miss a significant population that is at risk for tuberculosis so out of school youth require special attention. In addition, we found a high prevalence of *Non tuberculous mycobacteria* (NTMs), indicating a potential diagnostic challenge for the TB control program(4). The clinical relevance of this large burden of NTMs needs to be further explored.

We found a high TB disease incidence in adolescents (**Chapter 3**), indicating this population would be an appropriate target for TB vaccine trials. Having previous tuberculosis and a recent tuberculin skin test conversion were identified as risk factors for incident TB among this population. Schools are an ideal setting from which to recruit and follow these individuals but out of school youth are difficult to retain in follow up studies due to their high mobility. We also found a high annual risk of TB infection (**Chapter 4**) in this same population, indicating that TB vaccine studies that seek to prevent infection can also be considered in this population. Groups identified at being at higher risk for latent infection during our survey, including males and out of school youth; these

populations need special attention in TB programmatic prevention efforts. The association of a positive tuberculin skin test with a BCG scar as a risk factor for latent infection is less frequently observed after 10 years since BCG vaccination (5) but was present in this study. In a systematic review of the effect of BCG on TST measurements, the effect of BCG on TST was less after 15 years of age but BCG increased the likelihood of a positive TST(6). Interferon gamma release assays (IGRA) have a very high specificity that is independent of previous BCG vaccination and would have disentangled the contribution of BCG to TST reactivity(7).

The infrastructure and human research capacity created at our clinical research site in Siaya, were used to implement a Phase IIb (M72/ASO1_E vaccine) prevention of disease trial (POD) in adults aged 18-50 years (**Chapter 5**). This is the first vaccine trial globally to explore preventing latently infected individuals from progressing to active TB disease. The trial demonstrated overall vaccine efficacy, with differences observed in certain sub-groups in terms of protection; efficacy was found to be greater in younger study participants. The same age interaction has been described in a large trial in the BCG-REVAC cluster-randomised trial in Brazil where increasing age of those latently infected diminished vaccine efficacy(5). Our study was able to demonstrate efficacy both in the interim analysis (Chapter 5) and in the final analysis after 36 months of follow up (8).

We found a high TB incidence in infants indicating the site was attractive for TB vaccine trials among infants (**Chapter 6**). Conducting TB studies among infants was more challenging compared to adolescents, due to paucibacillary disease (9) which makes definitive diagnosis of TB more difficult and requires a reliance on clinical algorithms (10). In addition to high TB incidence, we observed that HIV infection was a risk for incident tuberculosis (Chapter 6). The study area has a high prevalence of HIV(11) with the attendant risk of transmission from mother to child. Thus a more robust and effective prevention of mother to child transmission program in this area would also reduce the risk of TB in children.

Endpoint definitions, needed for vaccine trials, are challenging as they rely on standardized clinical and radiological criteria to define TB disease (12-15). We observed poor inter-rater agreement among experts on radiological TB (**Chapter 7**), creating a need for better case definitions that can be replicated across trials. Results from medical officers and clinical officers performing clinical assessments were highly correlated to culture confirmed (definite) TB. This suggests clinicians can play an important role in endpoint determination, especially when they have an opportunity to review patient clinical presentations and radiographs. It has been suggested that better radiological imaging like CT scanning of the chest could improve radiological diagnosis and may be more reliable than chest X-rays (16). Improved biomarkers would also improve diagnostic certainty.

A dose finding TB vaccine trial in infants (Aeras 402) utilized the diagnostic and clinical trials capacity created by the TB incidence study (**Chapter 8**). The Aeras 402 candidate vaccine was

safe but induced lower CD4+ and CD8+ response rates in cytokine stimulating assays and Elispot assays in infants compared to those induced in adults. The failure to demonstrate an adequate immunological response in the dose finding study speaks to the challenges of developing TB vaccines targeting infants with immature immune systems and difficult to induce immunological responses. The possibility of boosting BCG with two or more different candidate TB vaccines and investigating whether this induces better immunological responses warrants further investigation.

Were the cohort studies necessary as part of preparing the site for TB vaccine trials

We identified two key reasons why conduct of the cohort studies and setting up the clinical trials site were critical: 1) TB notification data were insufficient to plan TB vaccine trial sample size calculations; and 2) The site had inadequate infrastructure and staff capacity/experience to conduct TB vaccine trials.

Notification data

Upon review of notification data from the National TB control program (NTP), TB burden estimates among adolescents and infants could not be obtained sufficiently due to missed cases. Some cases don't seek care in health services and there is limited sputum smear sensitivity for diagnosis of TB. There were underestimates on the burden of childhood TB due to incomplete identification of cases by passive case detection among adolescents (similar to what was found by van't Hoog et al) in the same study area (17) and as demonstrated in our prevalence study (**Chapter 2**). The TB control program uses only Ziehl–Neelsen (ZN) direct smears for TB diagnosis which have a sensitivity of 22-43% for a single smear (18). TB culture is not routinely available other than for retreatment cases and suspected multi drug resistance (MDR) cases.

The study area also has a high infant mortality (19, 20), malnutrition(20, 21) and HIV rates (22) with poor access to care and infant or pediatric TB diagnosis. For child TB diagnosis, the TB control program uses a score chart (10) which is partly confounded by malnutrition and HIV, and chest x-rays. Interpreting pediatric chest x-rays is challenging with a high intra and inter observer variability, with 39% sensitivity and 74% specificity(23). The inter observer agreement is also quite low (kappa -0.03 to 0.25) (24) and there was inadequate training of TB control staff to correctly interpret pediatric chest radiographs. Since young children cannot produce sputum easily, bacteriological confirmation with smear microscopy was impossible at the health facilities. In our study (**Chapter 7**), we also found a low inter rater agreement consistent with these findings demonstrating use of pediatric chest x rays for routine TB diagnosis is difficult.

Infrastructure

Diagnosing TB in infants requires facilities to conduct sputum induction and gastric aspirates and this capacity did not exist in the study area. Through the cohort studies, we were able to set up a case verification ward for overnight admissions of children with presumptive TB and collection of

samples for TB smears and culture. In addition, a concurrent malaria vaccine trial at the same site created a digital radiology unit at the site, greatly enhancing the quality of pediatric chest x-rays available for evaluation of TB.

To conduct the cohort studies, we ensured clinic space, including rooms for consenting, clinical procedures, data entry, quality assurance and quality control, phlebotomy, and appropriate laboratory space for initial processing, packaging and shipping samples to destination laboratories. The funding provided allowed the setting up of a clinical trials annex capable of conducting phase I-IV trials.

Prior to our studies, TB culture laboratories existed in Eldoret and Nairobi cities that are 150kms and 420kms respectively from Siaya. We upgraded our site such that a pre-existing biosafety level (BSL) 2 laboratory was converted to BSL 3, appropriate for conducting TB cultures. The technical expertise required to set up the culture laboratory, including monitoring cross-contamination, passing proficiency testing and getting accredited was provided by partners in the grant.

As part of the site development, an immunology laboratory was set up for peripheral blood mononuclear cells (PBMC) processing and storage. Setting up immunological laboratory capacity proved challenging, with local staff failing several proficiency tests on isolating quality peripheral blood mononuclear cells (PBMC) with adequate cell counts. After repeated training and proficiency the necessary skills were acquired and the capacity to implement high quality PBMC isolation established. Support from Aeras in setting up this immunology capacity including provision of training and proficiency testing was critical to success.

Training

Skilled staff that both understand the clinical methods for tuberculosis vaccine trials, and are experienced on good clinical and laboratory practices were not available at the site and conducting these epidemiological studies and implementing many procedures relevant to trials, unmasked many challenges that would have been subsequently encountered when conducting the actual trials including data entry errors, poor quality control and assurance, not complying with standard operating procedures and informed consent errors.

During the cohort studies, a transfer of knowledge occurred from a more experienced TB vaccine trials site (South African Tuberculosis Vaccine Initiative- SATVI) to our site. This included hands on training (getting our staff trained on administering and interpreting tuberculin skin tests, performing gastric aspirates and collecting induced sputum from infants), getting investigators trained at the University of Cape town on chest x-ray interpretation, and setting up a collaborative training with the Vienna School of Clinical Research where skills on clinical research, good clinical practice training, setting up standard operating procedures and establishing quality assurance and control systems for quality clinical data were established.

Some capacity needed to conduct clinical trials was already available including experience with developing protocols and submitting to ethics committees, working with electronic databases and reporting of all adverse events. In order to set up high quality TB vaccine clinical trials sites, all these moving pieces need to be brought together into a unified clinical trial site. There are few such sites in resource poor settings where the burden of tuberculosis is greatest. Developing such capacity requires participation in studies that would allow training and quality performance conducting the procedures critical for TB vaccine trials.

Implications for TB vaccine target populations

As discussed in the introduction (**Chapter 1**) modelling studies indicate that the greatest impact on reducing the TB epidemic will be from vaccines targeting adolescents and adults (1, 25) in order to accelerate the decrease in incident TB from 2% annually to 17% by 2025 leading to the goal of TB elimination by 2035 (less than 10 cases/100000 population) (26). Most TB transmission is from adults and adolescents with cavitary TB disease; reducing TB in this population will greatly reduce TB transmission (27).

Our study in adolescents (**Chapter 3**) confirmed they are an attractive group for pre-exposure vaccines before becoming latently infected with TB. We observed they are at high risk of infection as shown by high TST conversion rates in our cohort. We have shown that, despite lower incidence rates in Kenya compared to South Africa, our cohort is still a worthwhile site for TB vaccine trials(28-30). Due to the high annual risk of TB infection (ARTI), a post exposure vaccine after latent infection but before progression to TB disease would also have a high impact in this population, since studies have shown the highest risk of disease progression is within 1-2 years after infection(31). The M72/AS01E vaccine trial (**Chapter 5**) showed a higher efficacy in less than 25 year old adults. It's possible the majority of older adults have a farther distance from the infection event, making factors that lead to disease progression in them different from recently infected younger adults.

Even though the greatest impact would be with adolescents and adults, in our studies, infants had a higher TB incidence compared to adolescents (**Chapter 3 and Chapter 6**). Young children are sentinels of TB transmission and are the most vulnerable (32, 33). They have the highest risk of progression from TB infection to active disease, and the worst TB morbidity and mortality compared to older children and adults(33). Therefore, pursuing TB vaccine development for both populations is important. Even though the adenovirus vectored dose finding study (**Chapter 8**) induced an inadequate immunological response, BCG protects against severe forms of tuberculosis (TB meningitis and miliary TB) in infants(34). More effort needs to be put in identifying the mechanisms of this protection and building on it to develop better vaccines in infants.

Strengths and limitations

Strengths

The studies described in this thesis show how synergy between existing research capacities and additional funding for TB vaccine site development led to a well-developed trial site in a few years. The availability of the European and Developing Countries Clinical Trials Partnership (EDCTP) funding enabled investments in staff development and infrastructure that would not have been possible in the limited funding environment of a clinical trial. We leveraged the health and demographic surveillance system (HDSS) and the KEMRI/CDC Research and Public Health Collaboration infrastructure that had been in existence for years to set up the clinical trials site. Some of the research capacity included a liquid nitrogen manufacturing plant that made it easy to set up liquid nitrogen tanks for PBMC storage after processing. Centralised human resource and finance departments made hiring personnel, procurement and grant management quite efficient and allowed investigators to focus on the technical aspects of the setting up the studies and relevant infrastructure.

A pool of experienced staff emerging from a TB prevalence survey and resources acquired by the prevalence survey including a mobile CXR truck and generator were made available for the new TB vaccine site, enabling rapid set up of a survey among adolescents and leveraging community links that proved useful in recruiting from schools in the area.

The original grant had multiple collaborators that included epidemiologists and experienced clinical trialists. This enabled harnessing strengths in developing the protocols, training, reviewing study instruments and setting up study databases. One of the funders, Aeras, also provided direct support by sending monitoring teams to help with standard operating procedures (SOP) development and monitoring the quality of the clinical data collected.

The conduct of the studies obtained direct TB incidence estimates for adolescents and infants, providing estimates for a high burden setting to TB vaccine developers useful for sample size estimations. In addition, the preparatory cohort studies led to participation in multicenter trials of new TB vaccines.

Limitations

The funding provided by the European and developing countries clinical trials partnership (EDCTP) only allowed for a short follow up (a maximum of 2 years); longer follow up would have been more ideal to better document TB incidence (most TB vaccines studies would extend follow up time to minimize sample sizes) and a longer follow up time would have more accurately measured the retention that would be achieved in a 3 year study- a follow up time more related to what would be required in a TB vaccine trial. Another limitation is that we only used TST for TB infection which has limitations in cross reactivity with BCG and non-tuberculous mycobacteria (NTMs)(35).

An interferon gamma release assay (IGRA) is more specific for *M. tuberculosis* infection (36) and would have documented the incidence of TB infection better, but is more expensive and requires a laboratory with additional equipment to perform the IGRA. During follow-up after enrollment, only presumptive TB cases in infants received TST as part of the diagnostic work up. We missed a great opportunity to study the incidence of infection in the entire infant cohort by not doing an exit TST for all infants to determine the incidence of TB infection.

Due to lack of funding, no PBMCs were collected in the cohort studies. Such a collection at multiple time points would have allowed future immunologic studies to look at the differences between individuals with and without incident infection of TB or incident TB disease to see if there were any immunologic correlates of infection/disease.

Creating a BSL 3 TB culture laboratory and clinic space takes time, thus the cohort studies started and had to use an alternative laboratory to meet the timelines of the grant leading to several challenges including sputum contamination due to long incubation times before specimen processing, and decreased opportunities to use the cohort study samples to set up the quality standards needed for clinical trials. The laboratory was implemented while conducting the actual vaccine trials.

Implications for TB vaccine trials

There are a number of implications for vaccine trial design, sample sizes and future direction of TB vaccine trials from the work done as part of this thesis.

TB vaccine trial design

The high annual risk of TB infection (ARTI) in adolescents reinforces the need for prevention of sustained *Mycobacterium tuberculosis* (*Mtb*) infection (POI) trials that would utilize infection as an endpoint. The incidence of *Mtb* infection is approximately 8-10 fold higher than the incidence of active TB disease in a given population(37). Trials with a TB infection endpoint should be considered for phase II trials that demonstrate the possibility of clinical vaccine effects and form the basis for selecting vaccine candidates to advance to pivotal trials for disease efficacy endpoints.

A TB vaccine trial with a POI endpoint would be smaller due to the higher prevalence of infection compared to TB disease and have a short duration of follow up. Since majority of latently TB infected individuals do not progress to active disease and have only a 10% life time chance of developing TB disease(16), a POI trial would have to prove efficacy in this sub group to have meaningful impact on the TB epidemic(38). "The remaining 90% clear or control the infection and are not symptomatic or a source of TB transmission. If a vaccine had 90% or less vaccine efficacy against infection, it theoretically could be protecting only individuals who would have controlled the infection even in the absence of vaccination"(38). POI trials that proved efficacious would need to be seen as a stepping stone to POD trials that are accepted as the most rigorous endpoint for disease prevention and new TB vaccine registration(39).

A recent study in south Africa among TB uninfected HIV negative adolescents that looked at BCG revaccination or vaccination with a novel vaccine (H4:IC31) versus placebo to prevent infection showed 45% efficacy with BCG but not the novel vaccine against sustained latent TB infection (40). The risk of disseminated BCG disease (41, 42) is an important obstacle in HIV/TB co endemic areas for BCG revaccination but this findings create an opportunity to characterise the immunological mechanisms of protection. This promising result for BCG revaccination needs to be validated in populations near the equator where the burden of NTMs and helminths is much higher. In the Karonga study in Malawi, BCG revaccination offered no protection against incident tuberculosis(43-45). This study population also had a high NTM and helminth burden. There is evidence that helminths infection biases the immune responses towards Th2(46, 47) and high NTM infection rates(48) interfere with BCG protective responses causing lower efficacy. In our population, studies have shown a high helminth burden(49) and we found a large number of NTMs from TB cultures (**Chapter 2**). Nevertheless, the BCG revaccination finding reaffirm use of prevention of infection as a potential strategy for TB vaccine development and open up the possibility of reviewing the use of BCG revaccination in TB uninfected populations as an additional strategy for ending TB. This needs further evaluation in all high burden settings.

The high prevalence of non-tuberculous mycobacteria in the adolescent cohort raises issues of cross reactivity with *Mtb*(5). It has been suggested that a major reason for the poor efficacy of BCG vaccination against tuberculosis is common exposure to environmental mycobacteria. *M. avium*, *M. scrofulaceum* and *M. vaccae* exposure in mice blocks replication of BCG preventing vaccine protection against *M. tuberculosis*(50). In contrast, exposure to rapidly growing environmental mycobacteria in Malawi protected humans against tuberculosis and leprosy(49). Do NTMs boost immune responses to experimental products or will exposure to NTMS blunt the effect of experimental vaccines? The effect of abundant NTMs will need to be considered when assessing vaccine efficacy and evaluation of the immunological mechanisms of interactions should be evaluated to assess the potential impact on TB vaccine efficacy.

TB vaccine development is slowed by the lack of correlates of protection. Identification of correlates could substantially speed up development of other vaccines in the pipeline (currently 14 in the clinical pipeline). Biobanks from both of the recently concluded phase IIB studies(8, 40) will provide a plethora of data, potentially providing important insights on correlates of protection, and accelerating progress across the pipeline.

Prevention of TB recurrence (POR) after successful TB treatment has been proposed as a strategy to select TB vaccine candidates that would progress to prevention of disease trials(51). This would be a gating selection strategy where promising vaccine candidates which demonstrate efficacy against recurrent TB disease would be selected for large prevention of disease trials in the general population. There is a risk that TB recurrence might represent a sub population of susceptible individuals for which POR vaccine trials might have lower than expected efficacy. It still represents

an opportunity for smaller efficacy trials (smaller sample size and duration). Seventy to ninety percent of recurrences occur during the first year following treatment completion which is an efficient efficacy trial scenario. In our TB incidence study in adolescents, participants with a previous history of TB had a high risk of incident TB (**Chapter 3**). Other studies have shown a high rate of recurrent incident TB in patients with previous TB, with estimates ranging from 4.9% to 47%(52). A South African study found recurrence was 4 times higher than new TB disease(53).

Sample sizes

Despite the high number of TB cases found in adolescents and infants, only a minority of them had culture-confirmed TB, indicating that if this endpoint were strictly used, fairly large sample sizes would be needed. Prevention of pulmonary TB disease (POD) is a key public health goal and the most widely acceptable TB vaccine registration endpoint(51). This creates issues for phase 2b trials making them large, lengthy and costly, even in high TB burden areas. If a trial recruited both IGRA positive and negative individuals and had a 3 year follow up, using a culture-confirmed disease endpoint it would require 7000 participants. If it recruited only IGRA+ individuals, 3500 participants would be required with a 3 year follow-up.

Future trials

TB is mainly a disease of low-income settings, thus mobilizing resources to lower development costs for TB vaccine developers will enable the conduct of all phase trials including licensure trials (likely to be large and costly) that would lead to a new and effective TB vaccine. A dual pricing model where TB vaccines would be available to developing countries at a cheaper cost, would greatly enhance access and the impact on the TB epidemic(54).

Currently, the WHO in its latest report (2019) advocates for prevention of infection (POI) as one of the control strategies to achieve TB elimination. A planned recombinant BCG vaccination trial with VPM 1002 in infants in several African countries including Gabon, Uganda, Tanzania, Kenya and South Africa looks at POI as the primary endpoint. Discussions are under way between various stakeholders to capitalize on the first promising vaccine efficacy trial 100 years after introduction of BCG (M72/ASO1₂) to move it to efficacy trials. There is increasing hope to have a new licensed vaccine against TB in the next 5-7 years.

Conclusion

In conclusion, we found a high incidence of TB infection and disease among infants and adolescents making them an ideal target populations for TB vaccine trials. The M72/ASO1E TB vaccine efficacy will need further validation through a pivotal phase III efficacy trial. This fits well with the goal of advancing at least one TB vaccine candidate in adolescents and adults for prevention of disease. This will require adequate resources and formation of partnerships because no one organization alone can complete this and ensure access, given the challenge that the target markets will not guarantee a return on investment.

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