Discussion

The overall 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 thesis also documents the practical experience of site set-up and trial implementation in Kenya as well as the lessons for TB vaccine development following the successful trial of a new vaccine against Ebola virus. These questions have been addressed in TB cohort studies particularly among infants.

In the discussion, I review the principles for selecting a trial population, grouped into trial related factors and post-trial considerations using an infant population. Thereafter, I review epidemiological principles that can guide selection of other possible target populations considering disease patterns in endemic countries. This is followed by an appraisal of the chest radiograph as a diagnostic tool in trials. I discuss the role of Non-Tuberculous Mycobacteria in TB vaccine trials, as concerns TB diagnosis. Furthermore, I chronicle the lessons learnt in site development and examine the current pipeline of candidate vaccines targeting infants. Finally, I provide possible future perspectives, and provide a conclusion to the thesis.

The specific research questions addressed in this thesis are grouped thematically as follows:
The suitability of infants as a target population.

1. To assess TB incidence, post-neonatal mortality and cohort retention among infants.
2. To evaluate the chest radiograph (CXR) for its suitability as an endpoint for infant trials where paucibacillary disease is most frequent.
3. To determine the clinical relevance of non-tuberculous mycobacteria (NTM) isolated in sputum, given their ubiquity and their indistinguishable case presentation to TB disease.
4. 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.

Ad 1: Selecting a target population: Infants as a trial population. The selection of a target population requires consideration of multiple factors. I classify them into those related to trial implementation and those that concern post-trial roll-out.

1. Trial Implementation Related Factors

1a. Sample size calculations: Incidence, recruitment, retention, mortality

First, I compare trial efficiency of infant studies with adult/adolescent studies, computed using estimates of incidence, retention and mortality from an infant cohort study. The incidence of microbiologically confirmed TB among young children in our cohort study was high, at 0.28 per 100 person years of follow up. About 80% of the cohort was retained. We showed that in a superiority parallel arm TB vaccine trial, assuming definite TB incidence of 0.28 per 100 pyo (Nduba V, submitted), retention of 80%, vaccine efficacy (VE) of 50% (1, 2), 24,300 infants would need be recruited and followed up for three years.

In the recent M72 trial, TB incidence was 0.3% among LTBI positive adults who have higher risks of disease progression (1). A phase III licensure trial would need to include LTBI negative individuals

for validity, who have lower TB incidence, about 0.15% (3), with 80% power and 50 % VE. Based on these estimates about 50,000 healthy adults would be required. Further, larger sample sizes often require longer patient accrual times, multiple sites in different locations and complex logistics. All these inflate the trial costs (4). By recruiting 2900 infants in twelve months at a single site, we demonstrated the feasibility of accruing thousands of infants within a reasonable time frame. Therefore, we conclude that the feasibility and greater efficiency of infant trials has been shown.

1b. Mortality

About 7% of the infant cohort died over twenty-four months. The most frequent immediate cause of death was hypovolemic shock, while undernutrition was most frequently the underlying cause of death. Background mortality provides context for interpretation of vaccine efficacy. For example, Mosquirix®, in its phase III trial reduced the number and severity of malarial episodes but surprisingly had no impact on mortality (5). This has been attributed to local factors such as co-morbidities which drive mortality (6). In addition, as BCG has mortality benefits, all-cause mortality is an important secondary endpoint for infant TB vaccine trials. We conclude that baseline infant mortality estimates guide the comparisons of vaccine and control (BCG) arms in every site.

1c. Immunological factors: Vaccine design

MTBC is a complex pathogen, extensively evolved to evade and manipulate host immunity (7, 8). On the other hand, neonatal immune systems are underdeveloped, lack antigenic experience as a result of protected in-utero conditions, have poor immunological memory and heightened immunoregulatory responses to facilitate maternal co-existence (9, 10). Therefore, vaccinating adults has been more attractive. In addition, in high burden countries, maternal infections such as HIV, Malaria and helminths influence the development of fetal and neonatal immunity decreasing vaccine responsiveness (11).

2. Post-trial roll-out related factors: Vaccination platforms and impact on the epidemic

2a. Ethics

The high TB incidence among infants is further evidence of their higher susceptibility to infection after exposure, higher risks of progression to disease given infection and propensity to severe or disseminated disease (12). From an ethical perspective, infants are particularly vulnerable and need protection through an efficacious vaccine.

2b. Vaccination platforms

Vaccination of children is routine and largely accepted. BCG is the most widely administered vaccine (13). There are established cold chain infrastructure and implementation mechanisms even in poor countries. This infrastructure is currently not available for other target populations. They would likely require costly platforms to maximize coverage.

2c. Impact

As infants do not transmit disease, the public health impact of a neonatal vaccine would be delayed by about a decade (14), when they would become vulnerable. They are therefore secondary in TB control efforts. This has been viewed as a major limitation of infant vaccination. A long-term view is needed, as has been shown in some modeling studies, the rapid impact on TB burden of vaccinating adults with a post-exposure vaccine would plateau, while that of infants would continue to increase (15).

In summary, choosing infants is beneficial for trial efficiency, ethics and post -trial roll out; but has limitations of underdeveloped infant immunity and delayed impact on TB case load.

Additional considerations for selecting an adult target population. As infants do not transmit disease, adults and possibly adolescents must be included as 'immunisable' populations. I discuss some principles for selecting other target populations based on epidemiological principles and available data.

3. Trial implementation related factors

A way to minimize sample sizes, increase trial efficiency and feasibility, is to target high risk populations, contrary to the current paradigm which considers all persons living in high burden countries to have similar risks of disease acquisition. I discuss the rationale for this, provide real world examples and suggestions based on epidemiological data from high burden countries.

3a. 20/80 rule

The 20/80 rule in epidemiology states that 20% of the host population are responsible for at least 80% of the net transmission potential, as measured by the basic reproduction number R_0 (16). The consequence of heterogeneity in transmission of infectious agents is the commonly observed clustered distribution of infections and disease within the host population, such that few hosts are rapidly, frequently or heavily infected while the majority either evade the infection or rarely suffer infection. For example, one prisoner was responsible for 63% of incident TB cases in a Brazilian prison (17). These have been termed 'super-transmitters'. They combine high infectiousness with access to many casual contacts in congregant settings. It has been observed in different settings that household transmission accounts for <20% of cases (15). Surveys have shown areas of preferential transmission (18). Conversely, there are persons who despite repeated MTBC exposure remain IGRA/TST negative, termed 'resistors' (19, 20). This phenomenon has been observed in several diseases such as Ebola and HIV and the underlying factors are not always well characterized (21, 22). The reverse could also be true, where 20% of the population are particularly susceptible to acquiring infection and progressing to disease following casual contact. As correlates of risk have not yet been identified, using susceptibles as a target population has not yet been envisioned.

3b. Heterogeneity of disease burden

The 20/80 principle is exemplified in heterogeneity in TB burden based on spatial and social settings and interactions. They are discussed below.

3b1. Spatial heterogeneity

In high burden countries, 80% or more of cases can be confined to a particular geographical location. In a prospective cohort study of Multi-Drug Resistant (MDR-TB) cases and their household contacts in Peru, there was increased risk in specific neighborhoods proximal to a certain health centre and the authors argued targeted interventions at areas of concentrated risk would be more useful than a blanket approach. (23). Likewise, modeling transmission in 3rd most populated city in all of South America, Rio de Janeiro, showed only 6% of cases accounted for 35% of city-wide transmission (24).

3b2. Social heterogeneity

In the same vein, prisons, slums, public transport and health facilities have also been found to be hotspots of transmission (25-28). The prevalence rates in these areas are significantly higher than the general population regardless of economic status or local TB burden (29). In a recent vaccine modeling study using South African data, targeting miners and delivering the vaccine to them at the mines averted more TB cases compared to community targeted vaccination (30).

3b3. Wealth assortative mixing

A concept related to social hotspots is that of wealth assortative mixing (31) and is the basis of targeting the poor for TB vaccines and other control activities. It is hypothesized that the poor are more likely to contact the poor who in turn are more likely to develop disease and transmit it to other poor people. Such dynamics increase the R_0 and make it more challenging to control disease using a blanket, 'one size fits all' approach. In India, the poorest quintile of the population had a five-fold disease prevalence of that of the wealthiest (1% vs 0.2%). After accounting for poverty associated risk factors (alcohol and tobacco use, HIV, diabetes, low BMI), residual variance in TB rates was thought to be due to wealth assortative mixing. The authors recommended for equity reasons but also to improve efficiency to target the poor for interventions in order to optimize limited resources. It follows that the same should be selected as a trial population. There is precedence for targeted interventions in HIV (32-34), cholera (35, 36), and malaria (37).

3c. Post-trial access

Adult vaccination would likely struggle to achieve the high coverage observed with neonatal BCG vaccination. Vaccination platforms for adults would need to be created. Many high incidence countries have poorly funded health systems, overburdened by providing care and treatment. They may not be able to develop or sustain the logistical operation of delivering mass vaccination campaigns. Further, the acceptability and feasibility of adult vaccination has been taken for granted. As at publication, we could not find data on perceptions and acceptability of adult TB vaccination. Vaccine hesitance has now been recognized as one of the top ten global threats (17) and could undermine decades of efforts. This shows the difficulties that would need to be overcome with adult vaccination as a strategy for TB disease control.

In summary; there is heterogeneity in susceptibility to TB as well as transmission patterns. This should be taken into consideration when selecting the target population for efficient vaccine trials and consequently for disease control efforts.

Ad 2: The Chest Radiograph as a diagnostic tool for TB endpoints

We found low inter-rater agreement on abnormalities consistent with TB on the chest radiograph poor; kappa =0.14; (95% CI: 0.10 - 0.18). This and the frequently atypical picture in infants are limitations to its use. In another setting with high TB/HIV burden, abnormalities consistent with TB on CXR were predictive of culture confirmed TB among hospitalized children (38). One critical difference is that hospitalized children would be presenting with advanced disease and likely to have more classical features on which most readers would agree. Trials deploy active follow up strategies to detect every potential endpoint. This blunts the sensitivity of the CXR, as early disease is detected which causes non-specific and subtle radiological changes. Nevertheless, in the trial of MVA85A, odds of microbiologically confirmed TB increased two-fold when at least two experts agreed there was radiographic TB defined by classical features (39). Therefore, the CXR remains useful for endpoint detection when two or more experts agree on radiological TB. Diagnostics deploying minimally invasive samples, such as stool and urine, with high sensitivity and specificity, are needed for young children.

Automated reading of chest radiographs has been used to screen adult patients with presumptive TB before further diagnostic evaluation. It has been shown to have similar sensitivity but albeit lower specificity compared to expert readers (40, 41). It has not been evaluated in diagnosis in young children who have a different radiological pattern of TB. Therefore, its use in trials is presently not recommended.

Ad 3: Non-Tuberculous Mycobacteria (NTM): Clinical Relevance and Differential diagnosis for TB

NTM disease is clinically and radiologically indistinguishable from MTB disease (42). NTM disease does not respond to anti-tuberculous therapy. In paediatric studies, composite endpoints are used in the absence of microbiological confirmation. Unidentified NTM disease can therefore be a source of false positives, given the similar case presentation. However, we found that all NTMs isolated in sputum represented colonisation and not disease. There was also no association between NTM isolation in sputum and TST positivity. This could have been due to the small proportion of NTMs isolated. It was not a study objective to evaluate the immunological pathways of interaction between TB vaccines and NTMs. Their ubiquity in the environment and subsequent presence in the lung microbiome implies an immunomodulatory role. This should be evaluated in epidemiological studies and in future trials of both infants and adults with a view to define how they interface with host immunity. Induced sputum samples should be aliquoted and stored for ribosomal RNA sequencing to explore the role of flora in infection, susceptibility and disease. NTM based vaccine of *M. obuense* showed efficacy in HIV infected persons against TB (43). This is further proof of their immunomodulatory role.

Ad 4: Site development and the TB vaccine pipeline: Lessons learnt

We chronicled the history of the predominant TB vaccine trial site in Western Kenya. Some of the challenges encountered with respect to logistics and operations have been observed in other TB vaccine trial sites (44, 45). Many were unique to our specific settings such as the lack of reference ranges relevant to our population and immunology inexperience.

The TB vaccine pipeline calls for governments, particularly in high burden countries, to prioritize contributing to the research and development process, as happened with the Ebola virus outbreak of 2014-2016.

TB vaccine pipeline targeting infants: The review below shows candidate TB vaccines in which infant trials are planned, ongoing or have been completed.

Phase I trials

MTBVAC: MTBVAC is a live attenuated MTBC strain, first of its kind. Genes coding (*phoP*) for transcriptional factors associated with virulence have been deleted (46). A phase IIa dose finding, safety and reactogenicity study is underway in infants in South Africa, to be completed by December 2020. It could have potential for immunocompromised patients, as it showed efficacy despite hyper-attenuation (47).

HYBRID 56/IC31: Hybrid 56/IC31, is an adjuvanted vaccine currently in Phase I trials in South Africa for safety and immunogenicity in BCG primed infants. It contains Rv2660c, an antigen strongly recognized by cells of LTBI individuals. The adjuvant is IC31 which is a strong Toll Like Receptor 9 agonist.

Phase II trials

Phase II trials are the graveyard of new molecular entities particularly vaccines (www.bio.org/clinical-development-success-rates-2006-2015 accessed Dec 2019) (48-50). Only 31% of candidates progress, more frequently for lack of efficacy rather than safety. This contributes significantly to high research and development costs (48, 50). I examine the current status for infant vaccines.

MVA85A: MVA85A a modified vaccinia Ankara virus expressing antigen 85A (MVA85A), intended as a boost after BCG priming. The vaccine was safe and tolerable intradermal, in preclinical studies, and in the phase IIb trial in infants. However, it showed no efficacy against TB (51). The result was termed surprising based on preceding animal studies. It was later claimed that the animal efficacy results from these studies downplayed negative results. Apparently, macaques vaccinated with BCG and boosted with MVA85A had poorer outcomes comparable to those that received no vaccine (52). Additionally, there were doubts on the rigor of the pre-clinical studies (51, 53), which are said to be geared towards establishing safety for marketing authorization rather than providing evidence of efficacy. The authors refuted this (51). The vaccine has recently been reformulated as an aerosol. It was safe and immunogenic in a phase I study, for possible further investigation in phase II trials (51).

AERAS 402: Aeras 402 was a replication deficient serotype 35 adenovirus vectored vaccine containing DNA expressing a fusion protein of three MTBC Antigens, TB10.4, Antigen 85A, and 85B. It was

intended as a boost after BCG priming. Phase IIb trials were initiated in 2009 in Kenya, Mozambique and South Africa. After the dose-finding phase, the predefined immunogenicity target was not met, and the trial was terminated for futility (54). The vaccine was broadly immunogenic, however some of the reasons for failure were the immunodominance hierarchy elicited by adenoviral vaccination may not adequately mimic natural infection, leading to a non-protective immune response (55). Further development has stopped.

M72/AS01E: This is an adjuvanted vaccine expressing two Mtb 39A and Mtb 32A present in MTBC and BCG, combined with AS01E adjuvant. It has successfully completed Phase IIb trial in adults in Kenya, South Africa and Zambia, demonstrating 50% efficacy in preventing disease in LTBI individuals (Tait D, 2019). A phase II safety and immunogenicity study among infants was completed that demonstrated non-interference with the Expanded Program of Immunization (EPI) schedule, safety and satisfactory induction of CD4 T-cell responses. It is not clear whether phase IIb studies in infants are planned.

Phase III trials

VPM1002: VPM1002 is a urease C-deficient Listeriolysin expressing BCG vaccine strain. Listeriolysin facilitates the perforation of the phagosome membrane, allowing the release of recombinant BCG antigens into the cytosol of host cells, and activate appropriate Th1 and Th17 immune responses. The deletion of the Urease C gene acts synergistically with the Listeriolysin expression by creating an acidic milieu for its action. Its safety and immunogenicity have been assessed in a Phase II trial in South African infants (56). A phase III trial with the endpoint of preventing TB infection is underway in Kenya, Uganda and South Africa.

FUTURE PERSPECTIVES

Immunomics

The most suited trial population would take into account heterogeneity in individual susceptibility, and be well characterized by genetic, age and geographic factors. Immune responses would likewise accurately predict probability of progression to infection or to disease status after MTBC exposure or propensity to an inflammatory immune response which is more damaging to host tissues (7). In addition, a-priori knowledge of vaccine induced immunity would then be used to identify the target population most likely to benefit from vaccination. This would minimize sample sizes, minimize potentially harmful exposure to the investigational vaccine and maximize benefit, thereby increasing trial efficiency. After licensure, it would be cheaper and easier to create tailored platforms for vaccinating a small proportion of susceptibles, assuming that outside of neonates and adolescents, vaccination platforms would need to be created in the developing world.

This information is largely unavailable, and this remains an aspirational goal that could be achieved with the help of technology such as artificial intelligence and immunomics. Immunomics is a 21st

century approach that combines immunology, genetics, proteomics, transcriptomics and bioinformatics to characterize host -pathogen interactions that is particularly useful for vaccine development for complex pathogens such as TB (57).

Such an approach supports a rational approach to vaccine development based on identifying antigenic targets, Correlates of Protection and can unlock the keys to developing vaccines that confer lifelong protection.

CONCLUSION

We sought to determine the most appropriate population for vaccination, review the chest radiograph as a diagnostic tool for TB end-points in vaccine trials, the species and clinical relevance of NTMs in TB trials and the lessons learnt from a TB vaccine field site in the context of the Ebola outbreak. We found that selecting the trial population requires consideration of pre- and post-trial factors, and our studies provided data for such decisions. We found the CXR remains a useful diagnostic tool in the hands of two or more experts. In addition, we found NTM isolation in sputum of presumptive TB patients to represent colonisation and not disease, hence there is apparently no diagnostic dilemma. Finally, we described our twelve year experience developing a TB vaccine site and lessons learnt and concluded global efforts can be intensified and harmonized if a global coordinator such as the WHO were to take lead in the process of bringing a new TB vaccine to the market.

RECOMMENDATIONS

1. The most appropriate target population

Under a scenario of unlimited resources, vaccines targeting every high-risk population should be developed with the relevant endpoints. These include post-exposure vaccine for adults and adolescents (Prevention of Disease or Prevention of Recurrence), or Prevention of Infection for neonates. HIV infected persons would also benefit from a safe and effective vaccine as outside of infants, TB mortality is highest in this group. In the reality of the current funding scenario, vaccines targeting infants should be prioritized, due to the high incidence, the difficulty of diagnosing TB using current tools and the subsequent high case fatality of undetected and untreated disease. The second most urgent priority is adults in slums or other impoverished rural settings and prisons. From a bioethical standpoint, where the benefits outweigh risks, as with TB vaccine trials, and with proper oversight as is the current standard practice, research participation of prisoners can be informed, deliberate and voluntary (58-60). Further, experts have considered such that there is a tremendous need for scientifically sound research among prisoners (58). Vaccine acceptability studies should be done among adults in high burden countries to ensure high coverage post-approval. As was observed with the Ebola virus vaccine, funds should be committed to developing vaccination platforms for adults which are self-sustaining.

2. Greater investment in Research and Development by governments

Under the coordination of a global health entity such as the WHO, governments of high burden countries can be mobilized to contribute financially or in kind to vaccine research and development process. Many of these have reported unprecedented economic growth over the last decade. In kind or indirect contributions will also expedite the development process. For example, by removing barriers to research including costly tariffs on research equipment, including mandatory research training in the curriculum for health workers, thereby enhancing local capacity to conduct trials or by facilitating speedy ethical and regulatory review of research protocols.

3. The chest radiograph is still a useful diagnostic tool in trials

The chest radiograph should be included in infant trials with centralized, blinded, expert adjudication. New diagnostics, including point of care tests with high sensitivity and specificity that utilize minimally invasive techniques are needed. In addition to increasing end-point detection for trials, it will likely reduce TB mortality.

4. Limited autopsies to increase end-point yield in paediatric trials

Techniques for limited autopsies should be part of infant trials, as the causes of death were only identifiable in 31% of cases in our study. It is likely that there were TB deaths among these based on other autopsy studies in similar settings (61).

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