The possibility of evaluating vaccine candidates in a high burden population allows us to choose the more efficacious one that can be used in the future for the control of the disease in this population.

'And I don't know how to jump from the river side from today to the river side of tomorrow.'

_Garden_
Juan Ramon Jimenez

_From the series Dreamer_
Roberto Fabelo
Oil on canvas; 160 × 120 cm
INTRODUCTION

TB is a major global health problem particularly in developing countries (1). The BCG vaccine is the only licensed vaccine used to prevent TB. However, its efficacy is disputed (2). There is general agreement that it does provide protection against disseminated disease among children but its efficacy in preventing pulmonary TB in adults has varied widely from 0 per cent to 80 per cent. In recent years, there has been renewed interest in finding alternative vaccines against TB given a worsening of the TB epidemic due to the HIV pandemic and a reversal of gains previously made in reducing TB incidence (3).

DEVELOPMENT PLAN FOR TB VACCINE TRIALS

Preclinical development

The initial steps involve molecule discovery; most new vaccines comprise specific TB antigens. Some vaccine models have involved attaching these antigens to vectors while others have created fusion proteins mixed with adjuvants. Still others have modified BCG to create recombinant forms of the vaccine. One form of vaccine consists of a killed non-TB mycobacterial species with cross-reacting antigens with MTB. The next steps involve experimental testing of the product in animals looking at safety, immunogenicity, toxicity and efficacy, often with comparisons to BCG.

Phase I trials

The first phase of testing of the vaccine in healthy TB naive adult humans focuses on the safety and immunogenicity of the vaccine. Dose escalation testing may also be done at this stage. Some examination of the effects in BCG vaccinated individuals may also be carried out at this point.
Phase II trials
The primary goals of Phase II trials are to evaluate vaccine safety and immunogenicity in potential target groups. These groups include adolescents, infants, HIV positive individuals and latent TB infected individuals. A phase IIb study is a proof of concept study designed not only to focus on safety and immunogenicity but is also statistically powered to get an indication of potential efficacy using clinical endpoints so that a decision can be made about whether to proceed to a phase III study.

Phase III trials
This level of trial is designed to evaluate vaccine efficacy, and is conducted in the target group of interest. More recently, HIV negative adults have been prioritized as a target group since this would be a means of reducing transmission of the disease. HIV infected individuals, infants and adolescents are also important groups for vaccine testing because they are at high risk (HIV infected persons and infants) or precede a peak in incidence (adolescents). If efficacy is demonstrated at this stage, the application for licensure of the new TB vaccine product for public use can then occur.

Phase IV trials
As with any product, post-marketing surveillance for rare adverse events and the measurement of vaccine effectiveness would occur during this phase. Observational study designs may be used. Studies for new indications or evaluation in new target groups may also occur at this stage.

Clinical trial site development
Safety and immunogenicity testing may be done in a high or low TB burden population since only the direct physiological and immunological effects of a vaccine are being evaluated. However, since there is currently no immune correlate of protection for TB, a phase III vaccine trial site would need to be carefully selected in an area where TB is prevalent as the prevention of the occurrence of disease would be the prime measure of vaccine efficacy. Based on the experiences of the South African TB Vaccine Initiative (SATVI) of the University of Cape Town that has been conducting TB vaccine trials since 2001, the following requirements for a phase III vaccine trial site are proposed.
TABLE 7.2.1 Requirements for Phase III TB vaccine trial sites

**Epidemiological**

- Adequate rates of TB in the target populations (>0.5 per cent per annum).
- Ability to detect, investigate and document a very high proportion of significant health events which may occur for safety evaluation.
- Good surveillance systems with the ability to detect every case of TB which occurs.

**Clinical**

- Ability to diagnose TB infection and disease as accurately as possible including microbiological TB culture capacity.
- Competent clinical research team willing and able to prioritize TB vaccine studies.
- Adequate referral structures for detected cases of infection and disease.

**Immunology laboratory**

- Ability to at least process and dispatch blood specimens for analysis of immunogenicity.

**Ethics and regulatory authorities**

- Competent and efficient local and national ethical and regulatory authorities.

**General**

- Conducive environment: roads, telecommunications, power, security.
- Political stability and commitment.
- Established relationship with the local community.

**Epidemiological**

In order to measure efficacy, the incidence of the disease at a proposed trial site will determine the sample size. The higher the rate of disease, the lower the sample size requirements and vice versa. Internationally, few agencies are willing to fund TB vaccine trials, so funding for such trials is limited. Thus, an area with a high rate of disease would be more suitable for vaccine trials because the sample size required would better match the resources available to do such trials.

Health occurrences such as deaths, hospitalizations and TB disease events will need to be measured in clinical trials. The standard method of detecting these events through follow up visits would be applied in any trial. However, these events often occur in between visits. Surveillance mechanisms are thus needed to monitor death registers, hospital admissions and, health service evaluation and diagnosis of TB.
cases. A co-operative arrangement with a reasonably well functioning health care system would be a requirement.

An organized system for monitoring deaths either utilizing government based death registration systems or specially set up demographic surveillance areas are needed to monitor these. Regulators will have serious concerns not only about the ability of a trial site to detect deaths but also mechanisms to determine the cause of death. Regulators will be reluctant to register products where deaths have not clearly been shown not to be related to trial products.

**Clinical**

The capacity to diagnose TB with the implied laboratory infrastructure is needed. Sputum smear and culture and/or GeneXpert (Xpert MTB/RIF, Cepheid)) are important components of TB diagnosis. The diagnosis of TB in children is particularly difficult because the disease is pauci-bacillary. SATVI performs gastric washings and induced sputum procedures for smear and culture when investigating a child for TB. In addition, good quality X-rays are important in diagnosing paediatric TB. SATVI utilizes an expert paediatric radiologist panel to read the X-rays of infant TB suspects. A good clinical history and examination, and tests for TB infection such as the TST and more recently, the IFN-γ release assays are all important diagnostic aids. Once TB is diagnosed, a participant would need to be treated. Well-functioning experienced TB health care services should be available for the management of such patients.

**Training**

Staff involved in clinical trials are required to undergo training in Good Clinical Practice (GCP) or Good Clinical Laboratory Practice (GCLP). The capacity to conduct such training is needed. Protocol training needs to be conducted and training in study procedures is needed. Staff turnover is normal in any setting so the ongoing ability to provide training is an important component of any trial site. Thus, an in-house training unit or suitable accessible external training providers are needed for clinical trial sites.

**Immunology**

Although no markers of protection have been identified, the immune response is monitored as part of vaccine trials and work is already ongoing to investigate potential correlates of protection. Immune testing is still usually done to determine the impact of vaccination on human subjects even if it is still uncertain whether these responses will be protective. Thus, either an immunology laboratory with competent staff is needed to manage these tests or, alternatively, the capacity
to manage the storage and transport of specimens for immunological analysis is needed as these specimens often require special storage and transport.

**Ethics and regulatory authorities**

All trials require both institutional ethical approval and national regulatory approval. For the ultimate registration of any new product, all the trials prior to that point would need to have been managed in a way that is acceptable internationally. Any new TB vaccine would need to be used in many different countries. Local and national ethical and regulatory bodies thus have to ensure that trials are conducted in such a way so that not only international requirements are met but also local populations are protected where experimental products are being tested. Populations in developing countries are vulnerable to abuse and with the support of the necessary ethics and regulatory agencies, it is up to researchers to ensure that such abuse does not occur. Some vaccines are genetically modified products and many countries have specific regulations with respect to the import and export of such substances.

Efficiency is an important component of processing regulatory applications. Many regulatory agencies, due to inadequate resources, often take long to process trial applications. This would limit the ability of researchers to conduct trials in a timely manner and it would be a disincentive to sponsors to fund trials where the trial approval processes are lengthy.

**Quality assurance and quality control**

Most trials are subject to ongoing external monitoring and ad hoc audits by national and international agencies. The capacity to do internal monitoring is advisable to prevent problems from occurring as well as for early identification of problems. The regulatory environment for clinical trials is quite strict and both internal and external monitoring are needed to ensure conformity with regulatory requirements but these are also opportunities for growth and development of site personnel.

**Capacity development**

Large epidemiological cohort studies on TB involving 5,000–10,000 participants with 2-3 year follow up can be used to help develop Phase III clinical trial capacity. What this achieves is:

- It allows capacity to be built in the setting of a ‘mock trial’ with regard to e.g.
- Good clinical practice
- Data management
- Clinical procedures, e.g. tuberculin skin test (TST), gastric lavage, sputum induction
- Surveillance for TB and other important events
- Quality assurance and internal monitoring mechanisms
- It allows prevalence and incidence of TB infection and disease at the site to be accurately gauged.

General

Trials involve fieldwork to recruit participants and to conduct follow up visits. A good road infrastructure makes a big difference in facilitating such activities. While access to water, sanitation, electricity and telecommunications may be taken for granted, many developing countries struggle with these infrastructural issues particularly in rural areas. All are basic requirements in order for good clinical trials to be conducted. Trials cannot be conducted in an environment of political instability. Study timelines, visit schedules and after hours work would all be affected by instability and would undermine the success of any trial if these activities are severely affected in any way. Engaging with community representatives and stakeholders is an important part of trial site development particularly if many trials or long trials are to be conducted at a site. Language, culture, and power dynamics all need to be taken cognizance of where TB vaccine field trials are to be conducted.

THE SATVI EXPERIENCE

Research site selection

SATVI conducted a trial between 2001 and 2006, comparing the administration of BCG via the percutaneous route to the intradermal route and almost 12,000 infants were enrolled into this trial (5). SATVI’s research site is situated in the Boland Region of the Western Cape Province, South Africa. At the start of SATVI’s involvement in 2001, the total population of the study area was officially 266,825. Of the households, 89.8 per cent had access to a telephone, 84.3 per cent, access to electricity, 79.6 per cent, access to a flush toilet, and 91.5 per cent had access to water either in their dwelling or elsewhere on the site (Census 2001, Statistics South Africa).

The reported incidence rates of adult and childhood TB in the area were extremely high. In 2000, the reported incidence rate of smear positive TB for the whole area was 531 per 100,000 population (4). The area had a good primary health care and referral network. This was important to be able to run the studies in partnership with the public health services, relying on them to perform certain essential functions such as vaccination of...
trial participants and the provision of treatment for TB in any cases diagnosed by SATVI. There are around 25 ‘fixed’ clinics in the area and more remote areas are serviced by mobile teams. There are three ‘district’ hospitals, a referral hospital, and a dedicated TB hospital. Patients from the Boland who cannot be managed by the above are referred to one of three academic hospitals in Cape Town, some 100 km away. Reliable and efficient land and air ambulance services are available. All these factors led to this site being chosen as a field site for TB vaccine trials.

Recruitment and professional development of staff

Like many rural areas in South Africa, the Boland is not well supplied with physicians, nurses, laboratory technologists, and other categories of staff necessary for the execution of large field trials. Nevertheless, SATVI has been relatively successful in attracting staff through competitive salary packages and good employment conditions. An important factor was the appointment of Clinical Research Workers (CRWs) to support the clinical research activities at the site combined with a strong training department or Professional Development Programme as it is referred to at SATVI. Staff were trained in GCP to meet regulatory requirements and this enabled them to have a clearer insight into the special requirements of clinical trials.

Vital registration and morbidity surveillance

The regional Department of Health had good routine systems in place to monitor births (most of which took place in health care facilities) and to record deaths so that important events for TB vaccine trials were available for planning and surveillance purposes.

The surveillance system SATVI devised included the following elements.

Weekly lists of all admissions were obtained from the TB hospital, the referral hospital, and the three district hospitals in the study area. The names were cross-checked with the database of study participants. The medical files of study participants were then drawn and copied (consent for access to study participant medical records was obtained at study entry). These were later reviewed by one of the study medical officers. Participants with serious adverse events (SAEs) were followed up further if more data were required and any TB suspects were investigated in depth.

Because the routine mortality surveillance system can only provide very basic data, the system was augmented by study staff conducting verbal autopsies and collecting clinical records for any hospital admissions prior to death. A study medical officer, having reviewed all available information, assigned the ‘most likely’ main and underlying causes of death (6).
Tuberculosis surveillance and case verification

A reasonably sensitive but less specific public health system is available in the Boland for TB diagnosis and management in infants and young children. Some cases are diagnosed in hospital and, depending on which hospital, the grounds for treatment might include clinical signs and symptoms, tuberculin skin test (TST) results, gastric washing and/or extra-pulmonary body fluid TB culture results or biochemical changes, and chest radiography. Occasionally, diagnosis is based on more specialized investigations such as CT scan, ultrasound or histology. The levels of expertise of physicians in assessing patients, reading radiographs, and ordering treatment, range from newly qualified to experienced specialists. Many children diagnosed with TB are managed in clinics.

SATVI decided, in co-operation with the TB hospital, to equip and staff a ward into which children with suspected TB could be admitted, along with their primary caregiver, for a period of 48 hours. During this time the children had the following done: a comprehensive clinical history was taken and an examination was performed by a specially trained registered nurse. An HIV test was done. A finger prick rapid test (Abbott Determine©) was used and if the result was positive, a laboratory ELISA as well as an HIV-polymerase chain reaction (PCR) test were performed if the child was under 18 months old. A TST was performed. All children had two gastric washings and two induced sputa samples taken on consecutive days sent for TB culture, drug susceptibility, and biochemical and molecular speciation to exclude BCG disease. At one stage in the trial, blood samples were sent for TB culture but this was discontinued due to the very low yield. A chest radiograph was taken: routine antero-posterior (AP) and lateral films were taken and these were reviewed first by the medical officer in charge of the paediatric ward at Brewelskloof Hospital, and later by a panel of specialists experienced in the radiological diagnosis of TB in young children and infants.

Cohort retention

One of the reasons that the Boland was chosen as the site for the trial was that the population appeared to be fairly stable: there is seasonal migration due to the availability of work on the farms during the harvest and not at other times of the year, but labourers tend to return to the same farms, and there is no other large-scale migration documented. To make sure that this was the case, outmigration surveys were conducted at regular intervals. The outmigration rate in all surveys undertaken was low (cumulatively less than 5 per cent).
Monitoring and quality control

Standard operating procedures

Standard operating procedures (SOPs) were drawn up under the supervision of the quality assurance/control department. These were largely generic and work practice documents are drawn up when procedures are applied to specific studies. All documents are managed under a formal document control system which involves a special numbering system, version control, and document storage. Where necessary, specialists in the field are consulted. Once finalized, training of staff in the performance of the tasks described in the SOPs is done by study staff. All SOPs are periodically revisited and updated as experience is gained and inconsistencies become evident. SOPs covering the following procedures have been developed: recruitment, informed consent and enrolment, vaccination, vaccine management, vaccine reaction follow up and surveillance, adverse event surveillance and reporting, TB surveillance, case verification and review, outmigration surveys, database management, and needlestick injury management.

Internal monitoring

Initially, a senior research nurse with a strong background in clinical research and GCP was appointed as the study internal monitor, reporting directly to the principal investigator. With input from the data manager, a system was developed for monitoring a random 10 per cent sample of case report forms once a month and providing feedback on the results to the trial management for action. Fields were divided into critical and non-critical. If inconsistencies were found of greater than one per cent in critical or greater than 10 per cent in non-critical fields in any particular sample, this triggered a full audit of all records for that month. Many sponsors now use electronic data capture systems and staff are mainly involved in following up queries generated by these systems. Internal monitoring focuses now mainly on informed consent forms and source documents.

External monitoring

Community Advisory Board

A community advisory board (CAB) was constituted at the start of SATVI’s work and this was later expanded to broaden its representation. One of the site’s challenges is to maintain a fully functional community advisory board. The CAB has given input on a number of study related issues. The CAB has undergone GCP training covering trial management concepts but also infectious disease modules.
External Monitors and Audits

The site has routine external monitoring of all its trials and problems picked up through monitoring visits are dealt with. SATVI has undergone sponsor-arranged audits as well as audits by the national regulatory authority in South Africa, the Medicines Control Council. These have provided valuable developmental experiences for the site.

Institutional Review Boards/Independent Ethics Committees

All protocols and other relevant study documents are approved by the local University of Cape Town Human Research Ethics Committee and by sponsor institutional review boards (IRBs) in some instances. Trials of new TB vaccines also need to be approved by the South African Medicines Control Council (South Africa’s FDA equivalent). Annual reports are submitted to the IECs (independent ethics committees)/IRBs which include a summary of all serious adverse events. All serious adverse events are, in addition, reported to the local safety monitor or data safety monitoring committee and the sponsor’s chief medical officer, weekly. Six monthly reports on adverse events are required to be submitted to the medicines regulatory authority in South Africa. More rapid reporting is required for any serious adverse events.

Database management

Initially, a database manager designed databases using Microsoft Access for SATVI studies but more recently, more advanced databases provided by sponsors are being used with remote data entry as one mechanism of entering data. Where SATVI has designed the database, data entry is done on site in Worcester by dedicated data capturers and weekly backup copies of the database are kept both in Worcester and in Cape Town. Access to the databases is by password and this is restricted to the database manager, data capturers, study co-ordinators and the investigators.

CLINICAL TRIALS OF TUBERCULOSIS VACCINES

A number of vaccines have been developed in recent years (7, 8, 9) and some are going through clinical trials. Table 7.2.2 sets out those TB vaccines in human clinical trials at the time of writing.
<table>
<thead>
<tr>
<th>Status</th>
<th>Products</th>
<th>Product description [Citations]</th>
<th>Sponsor</th>
<th>Indication</th>
<th>Type of Vaccine</th>
<th>Target Populations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase III</td>
<td>Mw [M. indicus pranii (MIP)]</td>
<td>Whole cell saprophytic non-DTB mycobacterium [1–3]</td>
<td>Department of Biotechnology (Ministry of Science &amp; Technology, Government of India), M/s. Cadila Pharmaceuticals Ltd.</td>
<td>Whole cell, Inactivated or Disrupted</td>
<td>IT</td>
<td>—</td>
</tr>
<tr>
<td>Status</td>
<td>Products</td>
<td>Product description [Citations]</td>
<td>Sponsor</td>
<td>Indication</td>
<td>Type of Vaccine</td>
<td>Target Populations</td>
</tr>
<tr>
<td>--------------</td>
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<td>-------------------------------------</td>
</tr>
<tr>
<td>Phase II</td>
<td>M72 + AS01</td>
<td>Recombinant protein composed of a fusion of Mtb antigens Rv1196 and Rv0125 &amp; adjuvant AS01 [14–17]</td>
<td>GSK, Aeras</td>
<td>B PI</td>
<td>Recombinant Protein</td>
<td>Adolescents/ adults, nfants</td>
</tr>
<tr>
<td>Phase II</td>
<td>Hybrid-I+IC31</td>
<td>Adjuvanted recombinant protein composed of Mtb antigens 85B and ESATD6 [18–22]</td>
<td>Statens Serum Institute (SSI), TBVI, EDCTP, Intercell</td>
<td>P B PI</td>
<td>Recombinant Protein</td>
<td>Adolescents; adults</td>
</tr>
<tr>
<td>Phase II</td>
<td>VPM 1002</td>
<td>rBCG Prague strain expressing listeriolysin and carries a urease deletion mutation [23–27]</td>
<td>Max Planck, Vakzine Projekt Management GmbH, TBVI</td>
<td>P B</td>
<td>Recombinant Live</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>RUTI</td>
<td>Fragmented Mtb cells [28–32]</td>
<td>Archivel Farma, S.L.</td>
<td>B PI IT</td>
<td>Whole cell, Inactivated or Disrupted</td>
<td>HIV+ adults, LTBI diagnosed</td>
</tr>
</tbody>
</table>
### TABLE 7.2.2 Current TB vaccines in clinical trials (2011)

<table>
<thead>
<tr>
<th>Status</th>
<th>Product description [Citations]</th>
<th>Type of Vaccine</th>
<th>Target Populations</th>
<th>Sponsor</th>
<th>Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase I</td>
<td>AdAg85A Replication-deficient adenovirus 5 vector expressing Mtb antigen 85A [33–37]</td>
<td>Viral Vectored</td>
<td>Infants; adolescents; HIV+</td>
<td>McMaster University</td>
<td>Adjuvanted recombinant protein</td>
</tr>
<tr>
<td></td>
<td>Hybrid I+CAF01 Adjuvanted recombinant protein composed of Mtb antigens 85B and ESAT-6 [19–20, 38–40]</td>
<td>Recombinant Protein</td>
<td>Adolescents adults</td>
<td>SSI, TBVI</td>
<td>Mixed</td>
</tr>
<tr>
<td></td>
<td>Hybrid 56 + IC31 Adjuvanted recombinant protein composed of Mtb antigens 85B, ESAT-6 and Rv2660 [41–42]</td>
<td>Recombinant Protein</td>
<td>Adolescents adults</td>
<td>SSI, Aeras, Intercell</td>
<td>Mixed</td>
</tr>
<tr>
<td></td>
<td>HyVac 4/ AERAS/404, + IC31 Adjuvanted recombinant protein composed of a fusion of Mtb antigens 85B and TB10.4 [43–46]</td>
<td>Recombinant Protein</td>
<td>Infants</td>
<td>Infectious Disease Research Institute (IDRI), Aeras</td>
<td>Mixed</td>
</tr>
<tr>
<td></td>
<td>ID93/GLA-5E Subunit fusion protein composed of 4 Mtb antigens [99-100]</td>
<td>Recombinant Protein</td>
<td>Adolescents adults</td>
<td>Aeras</td>
<td>Mixed</td>
</tr>
</tbody>
</table>

(Source: http://www.stoptb.org/wg/new_vaccines/documents.asp which also provides updates of this table)
MEASURING SAFETY AND IMMUNOGENICITY ENDPOINTS

Assessing safety in human trials

Local, systemic, and serious adverse events would need to be measured in any safety vaccine study. Symptoms, signs, and laboratory measures are all evaluated in safety studies. Tables for grading severity have been developed by e.g FDA as mild, moderate or severe and these are used by many clinical trialists as it enables a standardised way of measuring such events. In TB vaccine trials, local reactions at the injection site are common and it would be important to measure how severe these are. The so-called ‘Koch’ phenomenon is also of concern. This hypothesised effect relates to where a person has TB disease or infection and the administration of TB antigens somehow aggravates the disease or brings about a severe reaction in those individuals who have been exposed. No trials to date have observed this phenomenon but this was observed when Robert Koch administered tuberculin to TB patients towards the end of the 19th century as he thought this was a potential treatment for TB.

An important issue is whether an observed adverse event is related to a particular product. The association of adverse events (AEs) to investigational products is judged by study investigators and, therefore, is subject to clinical judgement. The related adverse events form an important part of the profile of a vaccine and will impact on its acceptability by regulators and consumers.

Assessing immunogenicity in human trials

As biomarkers of a protective immune response against TB are not known, the immune response induced by a novel vaccine should best be termed “vaccine take”. Current assessment of vaccine take focuses on T cell immunity thought to be important for protection. Specific CD4 T cells able to produce IFN-γ are critical for protection against TB, as shown in congenital and acquired human immune deficiency, and in experimental models of mycobacterial disease. Therefore, specific IFN-γ production by CD4 T cells is measured in all current phase I or IIa trials of novel TB vaccines, either by an IFN-γ ELISPOT assay or by an intracellular cytokine staining (ICS) assay.

The IFN-γ ELISPOT detects production of IFN-γ by individual cells – each cell making the cytokine can be detected as a “spot” in a well (10). Each spot represents cytokine production by a single cell, following incubation of peripheral blood
mononuclear cells (PBMC) with specific vaccine antigens within the well. It is likely that most spots originate from specific CD4 T cells, although natural killer cells may contribute. In contrast, flow cytometric ICS assays detect IFN-γ production specific to CD4 T cells, following incubation of whole blood or PBMC with specific antigens (10). Multi-parameter ICS assays allow assessment of multiple complementary T cell outcomes. For example, CD4 T cell production of IL-2 and TNF, as well as of the pro-inflammatory cytokine IL-17 (11), cytokines that may contribute to protection against TB, may be measured within the same sample. Additionally, activation of CD8 T cells, which may also be important for protection, can be measured by the ability to produce IFN-γ, IL-2 and/or TNF, following specific stimulation.

ELISPOT and ICS assays are classic examples of shorter term assays, in which cells do not have time to proliferate, allowing a more quantitative assessment of outcome. Many other assays may be used to measure the T cell response (recently summarized in [12]). Some assays have longer periods of incubation (5-7 days), which may allow better assessment of cells that may require longer periods for activation, such as central memory T cells. The lymphocyte proliferation assay is a classic longer term assay: PBMC are pre-stained with a dye such as carboxy fluorescein succinimidyl ester (CFSE), incubated with specific antigens for 5-7 days, and proliferation of CD4 or CD8 T cells measured by flow cytometry (13). Every time the cell replicates it loses 50% of the original fluorescence intensity of the CFSE, allowing easy detection of expanded, specific cells.

Multiple variables determine assay success (summarised in [12]). For example, following blood collection, delay of incubation into assays or of PBMC isolation may compromise outcome. Also, the use of freshly isolated PBMC will allow greater sensitivity in measuring an immune response, compared with the use of thawed PBMC after cryopreservation. Selecting peptide vs. recombinant protein vs. whole bacterial (e.g., BCG) antigen, as well as the dose of the antigen, may significantly impact assay results.

**More on the induced immune response, and biomarkers of protection**

The exact nature of immunological mechanisms that mediate protection at different stages of MTB infection remain incompletely understood. T cell mechanisms other than those described above may be important, for example, cytotoxic activity, regulatory activity or activity of non-traditional T cells, such as γδ T cells. Innate cells, including macrophages, natural killer (NK) cells, and dendritic cells, may
be critically important. The dogma that antibodies, complement, or other host components are not critical players in immune control may simply reflect our lack of understanding of the complexity of the response to mycobacteria. Regardless, the emerging evidence of a well-balanced T cell response, which includes effector and regulatory arms, may be important for protection. Therefore, a quantitatively greater effector T cell response, following vaccination, is not necessarily ideal. Animal models of TB disease have taught us that specific IFN-γ production early after vaccination, particularly when determined in lung or local lymph node CD4 T cells, may correlate with protection (reviewed in [14]). However, results from multiple recent experimental studies caution against use of IFN-γ production as a sole vaccination-induced immune correlate of protection (15-17): in many cases, IFN-γ production merely reflects bacterial load and/or inflammatory status.

In humans, no vaccination-induced correlates of protection against TB, or correlates of risk of TB disease, using modern immunological tools, exist today. The TST reaction following BCG vaccination is a poor correlate of protection. Only one large study of biomarkers of protection against TB following BCG vaccination of newborns is currently ongoing, at the SATVI group in Cape Town, South Africa; preliminary results suggest that the classical T cell markers used to determine vaccine take, i.e., CD4 T cell production of IFN-γ, or even a polyfunctional CD4 T cell response (cells that make IFN-γ, IL-2 and TNF together, proposed to be a correlate of protection against intracellular pathogens), when measured at 10 weeks of age following newborn BCG vaccination, did not correlate with protection. Proliferation responses did not correlate with protection either. Other preliminary results suggest unbiased screening methods, such as DNA microarray analysis of gene expression, may yield patterns that are associated with protection – these approaches may ultimately allow discovery of novel correlates. These correlates may only be validated in placebo-controlled efficacy (phase III) trials of new TB vaccines, if efficacy is demonstrated.

**SAFETY AND IMMUNOGENICITY FEATURES OF NEW TB VACCINES**

A summary of key safety and immunological features of new TB vaccines currently in human trials is set out below (direct comparisons of vaccines is not possible because of different definitions and methods used for measuring adverse events and immunology outcomes).
AERAS-402

Safety

The safety profile of AERAS-402 developed so far (personal communication, Aeras Foundation) in adults consists of mild to moderate local injection site inflammatory findings with some cases of severe injection site pain, some moderate to severe constitutional symptoms, mild changes in liver enzymes, and mild to moderate changes in leukocyte parameters with some severe decreases in neutrophil count. It appears that there may be a dose-dependent increase in the frequency and severity of injection site reactions. There also appears to be an increased incidence of mild to moderate fever and myalgia in adult subjects receiving the highest dose level of AERAS-402 ($1 \times 10^{11}$ vp) compared to those receiving lower dose levels of AERAS-402. Aeras 402 has been described as well tolerated with no vaccine-related serious adverse events reported in an adult trial with this vaccine (10).

In the first study in healthy BCG-vaccinated infants, AERAS-402 provoked mild to moderate local injection site reactions. Mild to moderate constitutional symptoms related to AERAS-402, most commonly malaise, rhinitis, body temperature increase (fever), and fatigue, were also seen. A dose-dependent increase in the frequency of injection site pain, malaise, and fever was observed. Some changes in laboratory parameters were also attributed to AERAS-402, the most common (occurred in 2 or more infants) being hemoglobin decrease, ALT increase, and neutrophil count decrease. Most of the laboratory changes considered related to AERAS-402 were mild, although some infants had a moderate hemoglobin increase and one infant had a transient severe neutrophil count decrease. Instances of increased respiratory rate (tachypnoea) and rapid onset local erythema have been reported with a subsequent multicenter dose-finding trial in infants. The tachypnoea was reported as a severe unexpected serious adverse reaction (SUSAR) but all infants recovered within a short period of this reaction occurring. The erythematous reactions were self-limiting and no evidence of allergy was found. This vaccine was initially tested as a single dose vaccine then as a 2 dose vaccine (one month apart) and more recently, a third dose has been added 6 months after the first dose. There are 7 completed or ongoing trials of this vaccine in adults (35).

Immunogenicity

AERAS-402 is capable of inducing high levels of CD8+ T cells that respond to vaccine peptides in vitro in some volunteers. The CD4 response is measurable but less prominent. There is a general trend of increased immunogenicity with higher dose
levels. Polyfunctionality in T-cell responses has been an important finding with this vaccine. The CD8 responses were described as robust and durable in adults (18).

**GSK M72/AS01**

The M72 antigen is a fusion of 2 immunogenic proteins, MTB32A and MTB39A, formulated with GSK’s proprietary AS01 Adjuvant Systems (personal communication, O Ofori-Anyinam [GSK]). MTB32A and MTB39A are specifically expressed in BCG and MTB and induce proliferation and production of IFN-g by PBMC from PPD-positive donors. AS01 is composed of two immunostimulants, MPL and QS21, and a liposomal preparation. Following extensive preclinical evaluation, M72/AS01 has been tested in PPD-negative, PPD-positive, and HIV-positive adults (36, 37). It has now also been tested in infants and adolescents. A multicentre phase IIb efficacy trial in adults is planned.

**Safety**

Transient injection-site pain, redness, and swelling were local reactions reported for this vaccine. Constitutional symptoms such as fatigue and an influenza-like illness, headache or malaise have also been reported. These events generally decreased in severity or resolved within two days. No clinically significant abnormal laboratory results related to vaccination have been observed to date. There were no serious adverse events related to the vaccine that were reported.

**Immunogenicity**

The vaccine induces marked and persistent M72-specific humoral and polyfunctional cellular responses following administration of two doses. The second vaccine dose boosted the specific immune responses induced by the first dose. The profile of cytokine expression showed a significant frequency of M72-specific CD4+ T cells expressing two or more immune markers, among which are CD40L, IL-2, TNF-a, and IFN-g upon short-term in vitro stimulation. Additional characterization of the cellular response one week post vaccination on subjects living in endemic areas showed specific CD8+ T cell responses induced/boosted in subjects at seven days post dose 1 and to a lesser extent at seven days post dose 2.

**Hybrid-1-IC31 (H1IC)**

Ag85B-ESAT-6 (Hybrid-1) is the recombinant fusion protein of Ag85B and ESAT-6, developed and manufactured by Statens Serum Institute (Copenhagen, Denmark)
IC31® is a two-component adjuvant system developed by Intercell AG (Vienna, Austria), composed of the cationic polyaminoacid KLK and the oligodeoxynucleotide ODN1a.

Three phase I studies with this vaccine have been conducted in adults and two further trials are in progress, one in adolescents and one in HIV positive adults.

**Safety**

Vaccination to date has caused mainly local reactions such as transient soreness but also mild systemic symptoms.

**Immunogenicity**

The vaccine elicited strong antigen-specific T cell responses against H1 and both the Ag85B and the ESAT-6 components. In one study the volunteers from one group was followed up for immunogenicity after 2.5 year and the strong responses persisted through the 2.5 years, indicating the induction of a substantial memory response in the vaccine recipients (19, 20, 21).

**Hyvac4-IC31**

Ag85B-TB10.4 (Hyvac-4) is the recombinant fusion protein of Ag85B and TB 10.4, developed and manufactured by Statens Serum Institute (Copenhagen, Denmark) (personal communication, Ingrid Kromann, Statens Serum Institut). IC31® is a two-component adjuvant system developed by Intercell AG (Vienna, Austria), composed of the cationic polyaminoacid KLK and the oligodeoxynucleotide ODN1a (22). Four phase I studies have been conducted with this vaccine in adults with the latest being a trial in BCG vaccinated adults. Overall the vaccine has been shown to be well tolerated and immunogenic. No data on clinical trials for this vaccine has been published in the scientific literature at the time of writing. A trial in infants is being planned.

**MVA85A**

**Safety**

This vaccine which is administered intradermally commonly causes local reactions of redness and swelling initially, which then leads to scaling at the injection site which resolves within a few weeks. Less commonly, systemic reactions are experienced such as fever, headache, and cough (10, 23, 24). The safety profile in
subjects latently infected with MTB is comparable to that seen in BCG-vaccinated subjects (25). This vaccine has also be shown to be safe in HIV positive adults both on and off antiretroviral treatment who had CD4 levels >300 cells/mm³ (38). A trial in infants similarly showed this vaccine to be safe (39).

**Immunogenicity**

The main way in which the immunological response to MVA85A has been evaluated so far in the clinical trials conducted to date is the *ex-vivo* IFN-γ ELISPOT assay. MVA85A induced high levels of antigen-specific IFN-γ secreting T cells in BCG-naïve adults, and significantly higher levels in adults previously vaccinated with BCG (10, 23, 24). This boosting seems to be regardless of length of time between BCG and boosting with MVA85A (23). Immunogenicity results were similar in BCG-vaccinated adolescents and adults latently infected with MTB. In BCG-vaccinated adults, adolescents, children and infants, significant levels of antigen-specific T cells have been observed. In BCG-vaccinated adults in South Africa and the UK, BCG-specific CD4+ T cells boosted by MVA85A comprised multiple populations expressing combinations of IFN-g, TNF-a, IL-2, and IL-17, as measured by flow cytometry (10, 25). IFN-g-expressing and polyfunctional IFN-g+TNF-a+IL-2+ CD4+ T cells were boosted during the peak BCG-specific response, which occurred seven days after vaccination (25, 26, 27). A trial assessing interference with other routinely given vaccines in infancy showed the vaccine’s immune responses to be reduced by these routine vaccinations but the converse was not true (40).

**Efficacy**

This vaccine has been evaluated in a phase IIb trial in infants in South Africa for efficacy, the first new TB vaccine to undergo such an evaluation in infants. No evidence of efficacy against TB infection or disease was shown at the dose tested (41). While this result was disappointing, the trial provides a benchmark for further efficacy trials and many operational lessons were learnt (42).

**Mycobacterium vaccae**

This is the only new TB vaccine that has been through a phase III trial. Five doses of MV reduced definite TB by 39% in HIV positive adults and was immunogenic (28). This was consistent with immunogenicity from a preceding Phase II trial in Finland (29). Immunization was safe and had no adverse effects on HIV viral load or CD4 cell count in both trials. A broth production method for this vaccine
is now underway at Aeras. Interestingly, immune responses did not correlate with protection in the phase III trial (30).

**rBCGΔureC::Hly (VPM1002)**

VPM1002-specific human data are available from two clinical trials. In the first clinical trial conducted in Germany, healthy male Caucasian adult volunteers with or without pre-exposure to BCG were vaccinated with VPM1002 (N=30 + 30) or BCG (N=10 + 10) followed by a six month follow-up period. This study revealed that a single vaccination with VPM1002 up to 5x10^5 CFU was safe and well tolerated. No Serious Adverse Event occurred. VPM1002 was also shown to be highly immunogenic; it induced multifunctional CD4+ and CD8+ T cell subsets. With regard to the multifunctional CD8+ T cells, VPM1002 showed a trend of superiority over BCG at a comparable dosage. In addition it showed also a potential for a boost vaccination on a pre-existing immune response induced by BCG.

In the second clinical trial, 24 healthy male or female adults, all with prior exposure to BCG and predominantly from the indigenous African population, were vaccinated in South Africa. The 2 month safety data concurred with that of the German clinical trial, demonstrating that a single vaccination with VPM1002 up to a dose of 5x10^5 CFU was safe and well tolerated. (Personal communication – Leander Grode, Vakzine Projekt Management). This vaccine is being further tested for safety in infants and further trials in HIV exposed and infected infants are planned.

**rBCG30**

This vaccine is currently not in active development.

**Safety**

Similar responses were seen with rBCG30 as with BCG (20).

**Immunogenicity**

rBCG30 induced Ag85b-specific T cell responses involved both the CD4+ and CD8+ T cell components (31).

**RUTI**

The published results from a Phase I clinical trial report that “RUTI appeared to be well tolerated as judged by local and systemic clinical evaluation, though vaccine
dose dependent local adverse reactions were recorded. T-cell responses of blood lymphocytes to PPD and a number of antigen subunits were elevated, when compared with controls subjects (32). This vaccine is planned for use as a therapeutic vaccine against latent tuberculosis infection in combination with INH therapy (33).

**H56**

This vaccine is a variation of Hybrid-1-IC31 mentioned above but includes an additional antigen, Rv 2660, which is an antigen associated with latency. This vaccine has been developed to target individuals latently infected with TB. A phase I first in human trial has been completed in South Africa and no safety issues have been reported. Further trials are planned.

**ID93/ GLA-SE**

This fusion protein vaccine to be targeted at adults and adolescents has entered its first trial in the USA and this trial is ongoing. Depending on the results of this trial, further trials are planned in TB endemic countries.

**MTBVAC**

This live attenuated vaccine based on *M tuberculosis* has entered its first clinical trial in Europe and the trial is ongoing. No data on progress is currently available.

**CONCLUSION**

SATVI has set up a successful site for the testing of new TB vaccines which is ready to conduct Phase III vaccine trials. Multiple centres will probably be needed to conduct Phase III trials of TB vaccines and other TB vaccine trial sites in Africa and also other parts of the world have been developed. The lessons learnt by SATVI are valuable for sites currently in development and for any organizations/ institutions wishing to develop such a site.

There are a number of new TB candidates that have been developed and a number of these are in clinical trials in humans. Results thus far are promising both in terms of immunogenicity and in terms of safety profile but we will only know if any of these candidates will be able to mount an effective challenge to the TB epidemic at the Phase III stage.
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REFERENCES


