

TB vaccines need to be tested where the TB burden is the highest and amongst communities who need these vaccines most. At the same time, the highest scientific and ethical standards should be followed. Clinical trials of new TB vaccines in humans are necessary to establish safety, immunogenicity, and efficacy



**Untitled – Ivo Saglietti**  
Photography

'Fly child on the double  
moon of her breast.  
It, saddened by onions.  
You, satisfied.  
Never give way....'

***Lullaby of the Onion (Poem) – Miguel Hernández***

**Ivo Saglietti**

Genoa, Italy

Ivo Saglietti was born in 1948 in Toulon, France. He traded his movie camera for still photo equipment in 1978 and went to work in Paris for American and French agencies covering Latin America and the Middle East. In 1988, he began devoting his time to personal projects: taking photographs of Latin America, Kosovo, and the Mediterranean region's borders. He received the World Press Photo Award in 1992 and 1999 and the Enzo Baldoni prize in 2007 for 'Water and Oil', a photo essay on the Niger Delta. He lives in Genoa.

## CHAPTER 26

# Tuberculosis Vaccine Clinical Trial Site Development and Clinical Trials

Hassan Mahomed, Willem Hanekom, and Gregory D Hussey

### Introduction

TB is a major global health problem particularly in developing countries (1). The BCG vaccine is the only licenced 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 attached 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 stage involves experimental testing of the product in animals looking at safety, immunogenicity and efficacy, often with comparisons to BCG.

### **Phase I Trials**

The first phase of testing of the vaccine in healthy TB naïve 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 occur at this point.

### **Phase II Trials**

These evaluate safety and immunogenicity in potential target groups for the vaccine. These groups include adolescents, infants, HIV-positive individuals, and latent TB infected individuals. A Phase IIb study is a proof of concept study which not only focuses on safety and immunogenicity but is also statistically powered to get an indication of potential efficacy using clinical end points 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. For TB vaccine trials, infants are a priority. HIV-infected individuals and adolescents will also be important groups to test the vaccine in. If efficacy is demonstrated, then registration of the new TB vaccine product can be completed.

**Table 26.1** Requirements for Phase III TB Vaccine Trial Sites

<b>Epidemiological</b>
<ul style="list-style-type: none"> <li>• Adequate rates of TB in the target populations (&gt;0.5 per cent per annum).</li> <li>• Ability to detect, investigate, and document a very high proportion of significant health events which may occur for safety evaluation.</li> <li>• Good surveillance systems with the ability to detect every case of TB which occurs.</li> </ul>
<b>Clinical</b>
<ul style="list-style-type: none"> <li>• Ability to diagnose TB infection and disease as accurately as possible including microbiological TB culture capacity.</li> <li>• Competent clinical research team willing and able to prioritize TB vaccine studies.</li> <li>• Adequate referral structures for detected cases of infection and disease.</li> </ul>
<b>Immunology Laboratory</b>
<ul style="list-style-type: none"> <li>• Ability to at least process and dispatch blood specimens for analysis of immunogenicity.</li> </ul>
<b>Ethics and Regulatory Authorities</b>
<ul style="list-style-type: none"> <li>• Competent and efficient local and national ethical and regulatory authorities.</li> </ul>
<b>General</b>
<ul style="list-style-type: none"> <li>• Conducive environment: roads, telecommunications, power, security.</li> <li>• Political stability and commitment.</li> <li>• Established relationship with the local community.</li> </ul>

### ***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.

### **Clinical Trial Site Development**

Safety and immunogenicity testing may be done in any population since only the direct physiological and immunological effects of a vaccine are being evaluated. However, since there is 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) based at the University of Cape Town who have been conducting TB vaccine trials since 2001, the following requirements for a Phase III vaccine trial site are proposed.

#### ***Epidemiological***

In order to measure efficacy, the incidence of the disease in question will determine the sample size. The higher the rate of disease, the lower the sample size requirement 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 apply. 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 cooperative 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 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. Bacterial smear and culture are important components of TB diagnosis. The diagnosis of TB in children is particularly difficult because the disease is paucibacillary. SATVI does gastric washings and induced sputum procedures for

smear and culture when investigating a child for TB. In addition, there would be a dependency on good quality paediatric X-rays. SATVI utilizes an expert paediatric radiologist panel to read the X-rays of patients. A good clinical history and examination, and tests for TB infection such as the TST and more recently the IFN- $\gamma$  release assays are all important as diagnostic aids.

Once TB is diagnosed, a participant would need to be treated. Good experienced TB health care services should be available for the management of such patients.

### ***Training***

Staff involved in clinical trials are required to have training in GCP or GLP. The capacity to conduct such training is needed. Protocol training needs to be conducted. Training in 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.

### ***Immunology***

Although no markers of protection have been identified, the immune response is monitored as part of vaccine trials and work is ongoing to try and find correlates of protection. Immune testing is done to determine what impact the vaccine has in 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 immunology analysis is needed as these specimens often have special storage and transport requirements.

### ***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 that they not only meet international requirements but also protect local populations where experimental products are being tested. Populations in developing countries are vulnerable to abuse and it is up to researchers to ensure that such abuse does not occur, with the support of the necessary ethics and regulatory agencies.

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 researchers' ability to conduct trials in a timely manner and 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 cohort studies 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, for example,
  - Good clinical practice
  - Data management
  - Clinical procedures, e.g. 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 hour work would all be affected by environment 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

### How SATVI Selected Its Research Site

SATVI conducted a trial between 2001 and 2006 which compared the administration of BCG via the percutaneous route compared to the intradermal route and almost 12,000 infants were enrolled into this trial (5). It was in this context that SATVI chose its research site.

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

**Figure 26.1** A SATVI Research Technologist Determining Cell Counts



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 had access to electricity, 79.6 per cent had a flush toilet, and 91.5 per cent had water either in the 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 the more remote areas are serviced by mobile teams and 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 through to one of three academic hospitals in Cape Town, some 100 km away. Reliable and efficient land and air ambulance services are available.

### **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 appointing lay Community 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 in SATVI. Staff were trained in GCP to meet regulatory requirements and to enable them to have insight into the special requirements for 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 for 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). They 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 participants suspected of TB were investigated in depth.

Because the routine mortality surveillance system provided 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**

There exists a reasonably sensitive but less specific public health system for diagnosis and management of TB in infants and young children in the Boland. 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 cerebrospinal fluid TB culture results, other cerebrospinal fluid changes, and chest radiography. Occasionally, diagnosis is based on more specialized investigations such as CT scan or histology. The levels of expertise of physicians 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 cooperation with the TB hospital, to equip and staff a ward into which children suspected of having 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 examination performed by a specially trained registered nurse. An HIV test was done. We used a finger prick rapid test (Abbott Determine©) and if positive, this was followed by a laboratory ELISA as well as an HIV-polymerase chain reaction (PCR) test if the child was under 18 months old. A TST was performed. All children had two gastric washings and two induced sputa done on consecutive days sent for TB culture, drug susceptibility, and biochemical and molecular speciation to exclude BCG disease. At one stage in the trial, we were sending blood for TB culture but the very low yield prompted us to discontinue this. 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 a 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) have been drawn up under the supervision of the quality assurance/control department. These are 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 the training department and 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, morbidity/severe adverse event surveillance, TB surveillance, case verification and review, outmigration surveys, database management, and needlestick injury management.

### ***Internal Monitoring***

Initially, a senior research nurse with a good 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 feeding the results back to the trial management for action. Fields were divided into critical and non-critical. If inconsistencies were found 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. Later, a quality assurance manager and quality control officers were appointed to manage this function with the laboratory having a dedicated quality assurance staff member.

### ***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 has been to maintain a fully functional community advisory board.

### **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 an audit from 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 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 at both the sponsor and the host university which include a summary of all severe adverse events. All severe 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 back up 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, and the clinical manager.

## **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 26.2 below sets out those currently or previously in human clinical trials.

**Table 26.2** Current Tuberculosis Vaccines in Clinical Trials

Vaccine	Antigens	Vector/ formulation	Adju- vant	Developer
MVA85A	85A	Modified Vaccinia Ankara		H Mc Shane/ A Hill/ University of Oxford/Emergent
AERAS-402	85A, 85B and TB10.4	Replication defi- cient Adenovirus		Crucell N.V./ Aeras Global TB Vaccine Foundation
GSK M72/ AS01	MTB39 and MTB 32		AS01E	GSK
Hybrid-1	Antigen 85B–ESAT6		IC31	Staten Serum Institut (SSI)/ TBVAC
HyVac4-IC31/ AERAS-404	85B and TB10.4 (H4 antigen)		IC31	Staten Serum Institut (SSI)/ Aeras Global TB Vaccine Foundation
rBCG30	85B	Recombinant BCG which over- expresses antigen 85B		Marcus Horwitz/University of California, Los Angeles
rBCGΔureC:: Hly (VPM1002)	Multiple	A recombinant BCG that secretes listeriolysin and is urease C deficient		S Kaufmann, Max Planck Insti- tute for Infection Biology, Berlin, Germany/Vakzine Projekt Management (VPM)
<i>Mycobacte- rium vaccae</i>	Multiple	Whole cell myco- bacterial vaccine		J Stanford and G Rook, Depart- ment of Medical Microbiology, School of Pathology, University College and Middlesex School of Medicine, London, UK
RUTI	Multiple	Detoxified MTB cell fragments		PJ Cardona, Unitat de Tuberculosi Experi- mental Department of Microbi- ology, Fundació Institut per a la Investigació en Ciències de la Salut Germans Trias i Pujol and Universitat Autònoma de Barce- lona, Badalona, Catalonia, Spain/ Archivel Farma S.L.

## Measuring Safety and Immunogenicity End Points

Local, systemic, and serious adverse events would need to be measured in any safety vaccine study. Symptoms, signs, and laboratory measures are all

**Figure 26.2** SATVI Research Processing Clinical Trial Samples

evaluated in safety studies. Tables for grading severity have been developed by, for example, FDA as mild, moderate, or severe and these are used by many clinical trialists as it enables a standard 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' effect is also of concern. This hypothesized 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.

An important issue is whether an observed adverse event is related to a particular product. Relatedness of adverse events (AEs) to investigational products is judged by principal investigators and therefore is subject to clinical judgement. 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- $\gamma$  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- $\gamma$  production by CD4 T cells is measured in all current Phase I or IIa trials of novel TB vaccines, either by an IFN- $\gamma$  ELISPOT assay or by an intracellular cytokine staining (ICS) assay.

The IFN- $\gamma$  ELISPOT detects production of IFN- $\gamma$  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- $\gamma$  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, may be measured by ability of to produce IFN- $\gamma$ , 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 per cent of the original fluorescence intensity of the CFSE, allowing easy detection of expanded, specific cells.

Multiple variables determine assay success (summarized in (12)). For example, following blood collection, delay of incubation into assays or of PBMC isolation may compromise outcome. Also, use of freshly isolated PBMC will allow greater sensitivity in measuring an immune response, compared with 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  $\gamma\delta$  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, evidence is emerging that 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- $\gamma$  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- $\gamma$  production as a sole vaccination-induced immune correlate of protection (15, 16, 17): in many cases, IFN- $\gamma$  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, that is, CD4 T cell production of IFN- $\gamma$ , or even a polyfunctional CD4 T cell response (cells that make IFN- $\gamma$ , 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***

Reported adverse events related to AERAS-402 (personal communication, AERAS Global TB Vaccine Foundation), which is administered intramuscularly, so far include mild to moderate local injection site inflammatory findings (by far

**Figure 26.3** A SATVI Scientist Retrieves Biobank Samples for One of His Studies



the majority), some moderate to severe constitutional symptoms, mild changes in liver enzymes, and mild to moderate changes in leukocyte parameters. Some mild to moderate upper respiratory infections and other symptoms were judged to be related to the vaccine. No serious adverse events judged to be related to AERAS-402 have occurred thus far.

### ***Immunogenicity***

In volunteers, AERAS-402 is capable of inducing high levels of CD8+ T cells that respond to vaccine peptides in vitro. The CD4 response is measurable but less prominent. There is a general trend of increased immunogenicity with higher dose levels. Polyfunctionality in CD4 responses has been an important finding with this vaccine.

### **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- $\gamma$  by PBMC from PPD-positive donors. AS01 is composed of two immunostimulants, MPL and QS21, and a liposomal preparation. Following

**Figure 26.4** The Biobank in SATVI's Laboratory Houses Approximately 800,000 Samples in Liquid Nitrogen



extensive preclinical evaluation, M72/AS01 has been tested in PPD-negative, PPD-positive, and HIV-positive adults.

### ***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<sup>+</sup> T cells expressing two or more immune markers, among which are CD40L, IL-2, TNF- $\alpha$ , and IFN- $\gamma$  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<sup>+</sup> T cell responses

**Figure 26.5** Sample Processing at SATVI's Laboratory



induced/boosted in subjects at seven days post dose 1 and to a lesser extent at seven days post dose 2.

### **Hybrid-1**

No published data on clinical trials for this vaccine was found in the scientific literature at the time of writing.

### **Hyvac4-IC31/AERAS-404**

No published data on clinical trials for this vaccine was found in the scientific literature at the time of writing.

### **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. No serious adverse reactions have been reported that are related to this vaccine (10, 18, 19).

#### ***Immunogenicity***

The main way in which the immunological response to MVA85A has been evaluated so far in the clinical trials conducted to date is using the *ex-vivo* IFN- $\gamma$  ELISPOT assay. MVA85A induced high levels of antigen-specific IFN- $\gamma$  secreting T cells in BCG-naïve adults, and significantly higher levels in adults previously vaccinated with BCG (10, 18, 19). Immunogenicity results have been similar in BCG-vaccinated adolescents and adults latently infected with MTB. In BCG-vaccinated infants, significant levels of antigen-specific T cells have been observed. In BCG-vaccinated adults in South Africa, BCG-specific CD4+ T cells boosted by MVA85A comprised multiple populations expressing combinations of IFN- $\gamma$ , TNF- $\alpha$ , IL-2, and IL-17, as measured by flow cytometry. IFN- $\gamma$ -expressing and polyfunctional IFN- $\gamma$ +TNF- $\alpha$ +IL-2+ CD4+ T cells were boosted during the peak BCG-specific response, which occurred seven days after vaccination (18).

#### ***Mycobacterium Vaccae (21)***

This vaccine initially went through clinical trials as an immunotherapeutic agent against TB. However, a presentation at the International Union against Tuberculosis and Lung Disease Conference in Paris in 2008 showed promising data for the use of this vaccine as a preventative vaccine against TB in HIV-positive subjects. Formal publication of these results is awaited.

**Figure 26.6** SATVI's Project Site in Worcester is Located on the Premises of the Regional TB Hospital



### **rBCG $\Delta$ ureC::Hly (VPM1002)**

One clinical trial is in progress at the time of writing and preliminary results suggest that the vaccine is safe and well tolerated and that it induces good immune responses (CD4 and CD8) (personal communication—Leander Grode, Vakzine Projekt Management).

### **rBCG30 (20)**

#### ***Safety***

Similar responses were seen with rBCG30 as with BCG.

#### ***Immunogenicity***

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

### **RUTI (22)**

One Phase I clinical trial has been completed but no safety or immunogenicity data were available at the time of writing. This vaccine is planned for use as a therapeutic vaccine against latent TB infection in combination with INH therapy.

## 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 in other parts of the world are being 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 it will only be at the Phase III stage that we will know if any of these candidates will be able to mount an effective challenge to the TB epidemic.

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