World Health Organization

Report of the expert consultation on immunotherapeutic interventions for tuberculosis

Special Programme for Research & Training in Tropical Diseases (TDR) sponsored by
UNICEF / UNDP / World Bank / WHO

WHO Library Cataloguing-in-Publication Data

Expert consultation on immunotherapeutic interventions for tuberculosis
(2007 : Geneva, Switzerland).
Report of the expert consultation on immunotherapeutic interventions for tuberculosis Geneva, 29-31 January 2007.

1.Tuberculosis, Pulmonary - prevention and control. 2.Tuberculosis, Pulmonary - therapy. 3.Immunotherapy - utilization. 4.Antitubercular agents - therapeutic use. 5.Tuberculosis, Multidrug-resistant - therapy. I.World Health Organization. II.UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases.

ISBN 978 92 4 159583 4 (NLM classification: WF 310)

Cover design: Lisa Schwarb
Layout: Jocelyne Bruyère
Printed in France

TDR/IRM/07.1

Report of the expert consultation on immunotherapeutic interventions for tuberculosis

Geneva 29-31 January 2007

Acknowledgements

The report for the "Expert consultation to evaluate the potential roles of immunotherapeutic interventions for TB in TB and HIV high burden settings" was written by Dr GJ Churchyard assisted by Dr R Wallis, Dr J Levin, Professor G Kaplan, Dr P Onyebujoh and Dr M Vahedi. Professor Rook provided editorial comment. The meeting was chaired by Professor G Kaplan. The members of the respective working groups contributed to the recommendations.

Working group 1 (Therapeutic modulatory agents)
G Churchyard, R Hernandez-Pandos, S Jolles, D Lowrie, G Rook (chair), C Reading, H Ghalib, U Fruth, J Lazdins, A Odoula, R Peeling, S Nwaka, C Nacy, G Kaplan, J Stanford, J Frincke, H Ghalib.

Working group 2 (use of markers for TB treatment response)
A Aseffa, J Olobo, S Parida, E Sampaio, B Wallis (chair), A Ramsay, A Zumla, F Scano, J Kengeya, F Zicker, G Walzl.

Working group 3 (study designs to evaluate immunotherapies for TB)
G Bahr, L Rosa Brunet, J Levin (chair), C Stanford, F Zicker, D Maher, P Olliaro, M Gomes, MR Masjedi, C Reading.

TABLE OF CONTENTS

GLOSSARY

AED	Androstenediol
Ag	Antigen
ART	Antiretroviral therapy
BCG	Bacillus Calmette-Guérin – TB vaccine prepared from live attenuated bovine tuberculosis bacillus
CD8+	CD8+ T cells
CFU	Colony-forming units
COX2	Cyclooxygenase-II enzyme
CTL	Cytotoxic T lymphocytes
DHEA	Dehydroepiandrosterone
DNA	Deoxyribonucleic acid
DOTS	Direct observed therapy, short course
EBA	Early bactericidal activity
ESAT	Early secretory antigenic target
GMP	Good manufacturing practices – legal codification of sound quality principles used by the pharmaceutical and healthcare manufacturing industries to assure that products have the identity, strength, purity and quality that they purport to contain
HIV	Human immunodeficiency virus
Hsp65	Heat-shock protein 65
IFNγ	Interferon gamma
IgG	Immunoglobulin G subclass
IL-2	Interleukin-2
IL-4	Interleukin-4
IL-12	Interleukin-12
iNOS	Inducible nitric oxide synthase
IVIg	Human immunoglobulin administered intravenously
MDGs	Millennium Development Goals
MDR-TB	Multidrug-resistant TB
MVA	Modified vaccinia ankara
M. vaccae	*Mycobacterium vaccae*
MW	*Mycobacterium w*
M(X)DR-TB	Multi- and extensively drug-resistant TB
NLME	Nonlinear mixed effects
rh	Recombinant human
SLD	Second-line drugs
SSCC	Serial sputum colony counts

T cell	Precursors of T (lymphocyte) cells leave the bone marrow and mature in the thymus, hence their designation. T cells comprise two subsets distinguished by the presence on their surface of either CD4 or CD8 glycoproteins. T helper cells are further subdivided into Th1 and Th2 cells dependent on the cytokines they produce.
TDR	Special Programme for Research and Training in Tropical Diseases
TGF-β	Transforming growth factor beta
Th1 response	T helper cells secrete IL-1 and IFNg, enhancing cell-mediated responses and inhibiting both Th2 and humoral immune responses. Th1 cytokines (IL-2, IFNγ, TNF-α) activate macrophages and CTL and produce proinflammatory responses responsible for killing intracellular organisms.
Th2 response	Th2 cells secrete IL-4 and other cytokines which inhibit cell-mediated responses and enhance the humoral response.
TB	Tuberculosis
TNF-α	Tumour necrosis factor alpha
WHO	World Health Organization
XDR-TB	Extensively drug-resistant TB

Executive summary

The emergence of extensively drug-resistant tuberculosis (XDR-TB) has highlighted the need to strengthen TB control programmes and develop new treatment therapies. Immunotherapies have the potential to improve outcomes for all TB patients, including those with multi- and extensively drug-resistant TB (M(X)DR-TB). Immunotherapy may shorten TB treatments and improve the immunity of individuals cured by chemotherapy, potentially preventing recurrence. Currently none of the available candidate agents has proof of efficacy for use in drug-susceptible or drug-resistant TB, although some are registered for other indications in humans. Further development and evaluation of existing immunotherapy agents is required to find an effective agent that can be used adjunctively with chemotherapy.

The urgency of the situation demands accelerated clinical development which in turn requires surrogate markers of response to be defined. The most desirable surrogate markers would be expected to measure early during treatment a parameter that accurately predicts later relapse risk, regardless of the type or mechanism of action of the therapy under investigation. Rapid development of adjunctive immunotherapies for TB requires the development and use of both microbial and immunological surrogate markers. The characteristics and potential roles of such markers are discussed.

While we recommend fast tracking of immunotherapy agents for treatment of drug-susceptible and drug-resistant TB, these should be rigorous studies. The results obtained should be scientifically and clinically meaningful with particular regard to the safety issues of introducing immunotherapy in severely ill patients with compromised immune systems.

1 Introduction

The recent emergence of extensively drug-resistant tuberculosis (XDR-TB) has highlighted a systemic failure of health services; and the need to strengthen tuberculosis (TB) control and laboratory services and to develop new therapeutics.[1] Antituberculous chemotherapy remains the cornerstone of control. However, TB treatment is complex – requiring at least six months of treatment and associated with drug toxicities and drug-drug interactions. Current anti-TB drugs are unable to eradicate all *Mycobacterium tuberculosis* at sites of infection due to their relative inactivity against semi-dormant or persisting organisms, particularly those in lung granulomas. HIV coinfection is associated with increased recurrence of TB due to increased rates of reinfection and relapse. Treatment of multidrug-resistant TB (MDR-TB) and XDR-TB is much more complex and requires longer use of toxic, expensive, less effective second-line drugs. Advanced understanding of the immunopathogenesis of TB offers the potential for adjunctive immunotherapy for the treatment of drug-susceptible and drug-resistant TB. Immunotherapy is defined as the use of therapeutic vaccines or immunomodulation to shorten chemotherapy or reduce immunopathology. Adjunctive immunotherapy, in addition to chemotherapy, has the potential to shorten TB treatment and improve treatment outcomes of drug-resistant TB.

The scientific working group (SWG) of the TDR/WHO (3-6 October 2005) recommended that TDR and partners organize an informal consultation of experts to define research priorities and potential research activities that could enhance approaches on immunotherapy and immunomodulation agents for use in the management of TB. The SWG suggested that TDR, the Initiative for Vaccine Research (IVR) and partners could take a leading role in the evaluation of potential immunotherapy agents.

The justification for the meeting includes the need to find effective therapies for XDR-TB: an existing pipeline of immunotherapeutic agents could be moved into clinical trials relatively quickly with modest budgets.

2 Objectives and proposed outcomes

Objectives

- To discuss the potential utility of immunotherapy/modulation for optimizing and augmenting current TB chemotherapeutic regimens.
- To evaluate the utility of surrogate markers in indicating TB pathology and shortening TB trials.
- To discuss methodological constraints in designing studies on drug-resistant TB and in evaluating immunological interventions for TB.

Proposed outcomes

- Recommendations on the relevance of immunotherapy in optimizing current anti-TB chemotherapy and studies to evaluate these benefits.
- Recommendations on the utility of approaches to TB-detection relevant to immunotherapy and studies to evaluate these benefits.
- Recommendations on most suitable methods for determining the effects of immunomodulation in TB.
- Publication and wide dissemination of meeting report.

Dr Rob Ridley (Director of TDR) urged the group to think innovatively but to balance this with realism and strive for consensus. He requested honest reviews of immunotherapies, balancing their potential benefits and risks. Recommendations should include how to proceed with potential candidates and how to evaluate them effectively.

3 Report outline

The report summarizes plenary sessions and technical presentations which set the scene for the working-group discussions. The recommendations of the three working groups (immunotherapies, biomarkers and clinical-trial design) are presented. The agenda and list of attendees are in appendix 1 and 2 respectively.

4 Background presentations

4.1 Global TB control in 2007: achievements, challenges and the need for better tools (D Maher)

Globally, TB is the biggest cause of death from a curable or preventable infectious disease. Eastern, central and southern Africa have the highest TB rates which are fuelled by the HIV epidemic. The highest burden of TB occurs in countries with massive populations, such as India. The highest prevalence of MDR-TB among new TB cases between 1994 and 2003 occurred in Kazakhstan and other independent states of the former USSR, the Russian Federation, Israel and China. XDR-TB has been observed for some time but received more attention following the Tugela Ferry outbreak among HIV-infected individuals in South Africa.[2] The global problem of XDR-TB[1,3,4] is discussed further in section 4.2.

In 2005 the World Health Assembly set targets for TB control: to detect at least 70% of infectious cases and to cure 85% of detected cases. In China, directly observed therapy, short course (DOTS) reduced TB prevalence by 37% in less than a decade. Good progress globally was made under the DOTS strategy –TB case detection and cure rates increased to 60% and 84% respectively by 2005. TB incidence rates appear to be peaking globally and in Africa. However, there is no room for complacency. DOTS has not yet been expanded fully and is not of sufficient quality everywhere. Uncontrolled HIV-associated TB in Africa, MDR-TB in the former USSR and China, and the emergence of XDR-TB cause great concern. Weak health systems and services are compromising TB care. Affected communities are not aware, involved or mobilized.

The WHO-recommended Stop TB Strategy to reach the MDG 2015 targets includes:

- expansion and enhancement of high-quality DOTS
- addressing TB/HIV, MDR TB and other challenges
- contributing to strengthening health systems
- engaging with all care providers
- empowering people and communities with TB
- enabling and promoting research.

The Global Plan calls for a two-pronged approach to maximize TB control by using existing technologies optimally and developing new tools. This requires a TB research "movement" to reach the 2015 targets. The estimated cost of implementing the Global Plan is US$ 56 billion; currently there is a US$ 31 billion shortfall. The Plan indicates projected funding needs for the development of new drugs (US$ 4.8 billion), vaccines (US$ 3.6 billion) and diagnostics (US$ 0.5 billion), but not for upstream research by basic scientists (innovation) or for research on immunotherapies. Money has been allocated for the development of new drugs (US$ 4.8 billion), vaccines (US$ 3.6 billion) and diagnostics (US$ 0.5 billion), but no funds have been allocated for upstream research by basic scientists (innovation), operational research or immunotherapies.

4.2 Update on the activities of the WHO taskforce on XDR-TB (P Nunn)

XDR-TB is defined as resistance to isoniazid and rifampicin in addition to a fluoroquinolone and one of the injectable drugs (kanamycin, amikacin and capreomycin). It is a major global threat to TB control. By 1 May 2007 37 countries had reported confirmed cases of XDR-TB. Of the estimated 420 000 MDR-TB cases worldwide, the majority occur in China, India and independent states of the former USSR, which is where the bulk of XDR-TB could therefore be anticipated. The diversity of strains suggests that XDR-TB has emerged due to a systemic failure of health services.

Treatment options for XDR-TB are severely restricted and treatment outcomes are poor. Very high mortality was reported in the Tugela Ferry outbreak in Kwa-Zulu Natal, South Africa.[2] All of the 53 patients reported were HIV-infected; 52 of the patients died on average within 16 days of taking a sputum specimen, including those on antiretroviral therapy (ART). This contrasts with reported cure rates of around 30% in Latvia, which has low levels of HIV.

A global task force, convened in response to the XDR-TB threat, made the following recommendations:

- strengthen control of TB and HIV
- scale up programmatic management of M(X)DR-TB to prevent XDR-TB
- strengthen laboratory services for adequate and timely diagnosis
- expand M(X)DR-TB surveillance to understand better the magnitude and trends of drug resistance
- foster sound infection control
- develop new tools.

The MDR- and XDR-TB response plan for 2007 was launched on 22 June 2007. This event marked the transition from crisis management to mainstream response. As part of the plan, it is likely that a research and development meeting will be held during the World Lung Health Congress in Cape Town in November 2007. The authors of this report recommend that this opportunity is used to place immunotherapy on the research and development agenda.

4.3 TB vaccines: current candidates and development plans (U Fruth)

TB vaccines share common objectives with some of the proposed immunotherapies. The vaccines are intended to induce or boost protective immunity against acquisition of infection in neonates and infants, and prevent progression from infection to disease or attenuate TB disease in adults. Immunotherapy strives to restore protective immunity and/or reduce immunopathology.

Infant vaccination with BCG protects against severe childhood manifestations of TB for those up to 10 years old. However, BCG-induced immunity wanes over time and it is ineffective to boost adolescents and adults previously exposed or vaccinated with BCG. It is unethical to stop infant BCG vaccination, so the aim is to develop a booster vaccine that improves rather than replaces it.

TB vaccine development is highly empirical. A number of vaccine strategies are being evaluated, including those listed below.

- Improved BCG, with over-expression of protective antigens or reconstitution of genes that have been deleted from BCG.
- Attenuated *M. tuberculosis*, which have had metabolic or virulence genes made inactive.
- Adjuvanted protein subunit vaccines, including peptides and DNA vaccines, which use secreted or empirically selected antigens such as Mtb32/Mtb39, Ag85B-ESAT-6 and Ag85B/TB10.4 fusion proteins.
- Live vectors, such as modified vaccinia ankara (MVA), adenovirus and salmonella, which express antigens such as 85A, 85B and TB10.4.
- Non-protein antigens such as those binding to CD1-binding molecules or $\gamma\delta$ T cell receptors.

The Global Partnership to Stop TB Working Group on New TB Vaccines has several objectives.

- One safe, effective, licensed vaccine to be available at reasonable cost by 2015.
- Maintain and improve BCG vaccination programmes.
- Conduct discovery and translation research.

- Facilitate preclinical development.
- Build capacity at vaccine-trial sites.
- Ensure availability of vaccine-production capacity and ability to scale up.
- Perform clinical trials.
- Develop an enabling infrastructure.

Under optimum conditions the first generation vaccine for infants will be available by 2013-2015, and a post-infection vaccine by 2018.

The Stop TB Partnership Working Group on New TB Vaccines provides a forum for discussing vaccine-development issues; is an impartial broker among stakeholders; and facilitates consensus on protocol design and immunological and clinical endpoints. The working group includes regulators from countries which have endemic TB. The establishment of a TB immunotherapeutics working group, modelled on the vaccine working group, might facilitate the evaluation and implementation of immunotherapeutics.

5 Immunotherapy for human TB – why do we need it? *(G Rook)*

Immunotherapy may have multiple roles in TB treatments: improving success rates for treatment of M(X)DR-TB; shortening treatment-times for drug-sensitive TB; and improving the immunity of individuals cured by chemotherapy, thereby preventing recurrent disease (whether through true relapse or reinfection).

Current candidate TB immunotherapies are at different stages of clinical development. Several immunotherapy constructs that are active in mouse TB models (with or without chemotherapy) have been produced to good manufacturing practice (GMP) standards but have not yet been tested on humans. A number of immunotherapies have been evaluated on humans, although not all have been studied in patients with TB.

5.1 Immune protection and immunopathology

The nature of the immune response to TB infection determines whether it is protective or will result in TB disease. A model has been proposed to explain the immunological responses that result in either protection or disease, which may be manipulated by immunotherapies or vaccines (Fig. 1).[5]

Protective immunity comprises bacteriostatic and bactericidal components. Both may be expressed within a single infected host, at different times, or in different parts of affected organs. Although a bacteriostatic response may provide transient protection from an invasive pathogen it does so by creating dormancy, a source of recurrent infection that can be difficult to eradicate. The challenge for immunotherapy is either to convert a predominantly bacteriostatic immune response into a bactericidal response, or to modify the bacteriostatic response so that it no longer interferes with chemotherapy (section 6.2).

Immune protection results from phagocytosis and killing, or controlling the growth of, mycobacteria by activated macrophages and lymphocytes. The cellular immune response dominated by antigen-specific T cells that produce gamma interferon (IFNγ) and are cytotoxic towards infected cells is referred to as a type-1 (bactericidal) cellular response. The main macrophage-activating factor, IFNγ, is essential for immune protection though it can mediate a bacteriostatic rather than bactericidal response. Antigen-specific cytotoxic T lymphocytes (CTL) play a central role in protective immunity, possibly by granule-mediated lysis of infected macrophages, resulting in death of the contained organisms. Thus bactericidal immunity is probably the classical Type 1 IFN-γ-mediated response, plus CTL. Most immunotherapy agents have a direct or indirect effect on cytokinese-producing and cytotoxic T cells (Fig. 1).

An immune response with an excessive proportion of antigen-specific T cells that produce Interleukin-4 (IL-4) (type 2 cellular response) is immunopathological rather than protective. In spite of granuloma formation this immune response cannot eradicate the infection (bacteriostatic immune response). The IL-4 stimulates macrophages to produce transforming growth factor beta (TGF-β) and both down-regulate the CTL response. The detrimental effects of IL-4 are due to reduced microbicidal activity of macrophages, increased cell infiltration, toxicity of tumour necrosis factor alpha (TNF-α) and inflammation that leads to necrosis, increased collagen, and activation of TGF-β that leads to fibrosis. TGF-β is produced by macrophages and T regulatory cells and decreases T cell proliferation and inhibits IL-2, IFNγ, TNF-α, inducible nitric oxide synthase (iNOS) production and pro-inflammatory responses.

Granulomas result from the sequential recruitment of cells to the site of TB infection which creates a physical barrier that contains infection. The hostile microenvironment within the granuloma, in which oxygen tension, pH and micronutrients are reduced, induce mycobacteria to enter into a latent state

by profoundly altering their metabolism, biosynthesis and replication. Granulomas have both protective and detrimental effects, particularly when they liquefy. Macrophages migrating into the cavity are permissive and support the growth of organisms.

A dominant Th2 immune response produces immunopathology. The resultant inefficient killing of mycobacteria, central necrosis of granuloma (caseation), destruction of lung tissue and rupture of cavities into the bronchi spread TB. Immunopathology is more likely to occur when TB infection involves a Th1/Th2 response. Environmental mycobacteria, which occur more commonly in developing countries, prime Th1 and Th2 responses. Helminth infections further promote a Th2 response. In this setting, TB infection results in the production of IL-4 superimposed on an existing Th1 cell response. This compromises cell-mediated immunity to TB by reducing microbicidal activity, inducing inflammation and increasing pulmonary fibrosis.

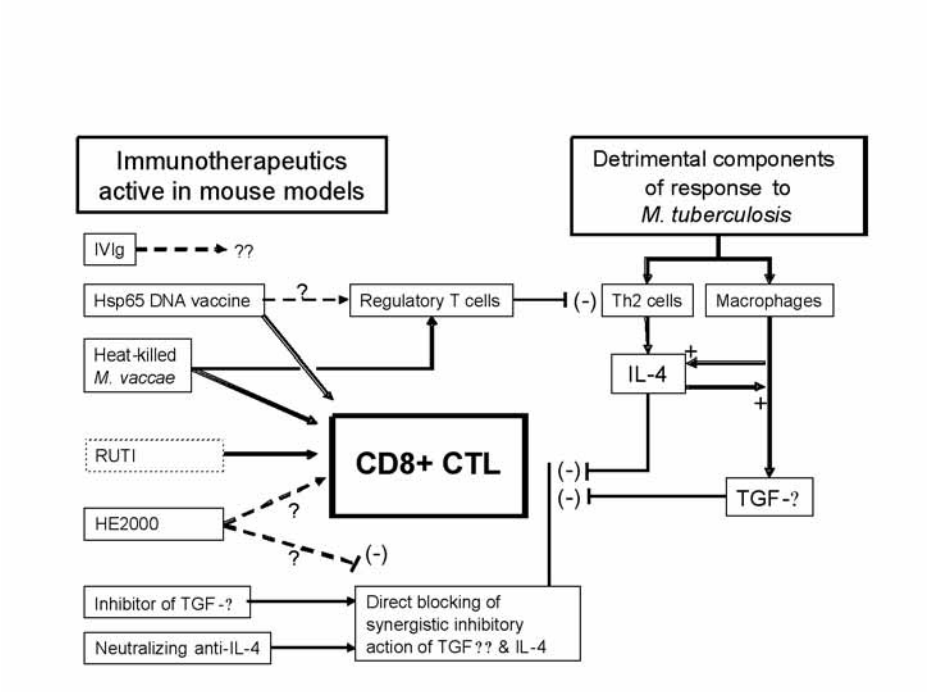

Figure 1 Mechanism of action of immunotherapeutics[6]

6 IMMUNOTHERAPY AGENTS

Immunotherapy aims to "realign" or improve the immune response either by promoting protective (Th1) immunity or by blocking harmful immune (Th2) responses. It is not necessarily better to induce more protective immunity as TB patients have a large Th1 response in their lungs. Furthermore, boosting the Th1 responses may induce systemic release of Th1-associated cytokines resulting in necrosis of TB lesions (Koch's phenomenon). It may be better to optimize the Th1 response by down-regulating the Th2 response.

There are two types of immunotherapy agents. Some enhance protective immunity and/or down-regulate Th2 activity; others facilitate access to, or activity of chemotherapeutic agents in, the bacilli by disrupting bacteriostatic pathways or fibrosis.

The mechanism of action of the available agents is summarized below. The plenary overview of immunotherapeutic agents was presented by Professor Rook. Those who gave the technical presentations for each agent are named in parentheses.

6.1 Agents that enhance protective immunity and/or down-regulate Th2 activity

Immunotherapeutic agents can be classified according to whether they have been studied in mice (Hsp65 DNA vaccine, anti-TGF-β, anti-IL-4), humans (human immunoglobulin), or both (killed *M. vaccae*, HE2000, rh-IFNγ, rh-IL-2). The mechanism of each is summarized below.

6.1.1 Agents studied in mice

1. DNA vaccines (D Lowrie, C Nacy)

A number of DNA vaccines have activity in TB-infected mice. Apart from 10-antigen mix, the other DNA vaccines (which express Hsp65, IL-12; Ag85A, PST S3, IL-12; Ag85B; Hsp70/CD80; ESAT-6 in flu vector) show a one to three log increase in bacterial clearance in comparison to untreated mice.

DNA vaccines encoding a mycobacterial (*M. leprae*) stress protein (Hsp65)[7] or Hsp70 fused to CD80 (Chen et al, unpublished data) are therapeutic when administered to tuberculous mice. A DNA vaccine expressing ESAT-6 in a flu vector is effective as adjunctive therapy when administered with chemotherapy. Immunotherapy with Hsp65 as an adjunct to chemotherapy is associated with more rapid and efficient response to treatment of MDR-TB in mice.[8] There is synergy between chemotherapy (moxifloxacin) and DNA vaccine in BCG-immunized, TB-challenged mice. DNA vaccination at the end of chemotherapy has a sterilizing effect in mice.[9] Monkeys immunized with Hsp70/CD80 before being infected with TB survived; those that received nothing or the BCG vaccination, died (Chen et al, unpublished data).

The therapeutic effect of DNA vaccines is associated with a switch from a predominantly type 2 to a predominantly type 1 response. DNA vaccines enhance IFNγ production and CD8+ CTL, which can lyse macrophages infected with M. tuberculosis, down-regulate IL-4 production and eliminate persisting organisms.[7] Plasmid DNA expressing IL-12 also has an immunostimulatory "adjuvant" effect that would further promote the shift from a Th2 to a Th1 response.[10]

Clinical trials of DNA Hsp vaccines in humans have shown them to be safe. BCG Hsp65 fused to E7 viral protein has been used in a human papilloma virus vaccine and rh-Hsp70 with polyvalent antigen used in a vaccine for genital herpes. Reports that Hsp may exacerbate rheumatoid disease are not confirmed.

In summary, DNA vaccines may be therapeutic or work as adjuncts to chemotherapy to enhance bacterial killing, reduce pathology, eliminate organisms that persist, and protect against reinfection. DNA vaccines are equally effective against MDR-TB.

2. Inhibition of TGF-β (R Hernández-Pando)

Inhibition of TGF-β with β-glycan (soluble Type III TGF-β receptor) is associated with increased expression of IFNγ and IL-2; strong down-regulation of IL-4; and significant reduction in bacterial counts in the absence of chemotherapy.[11] However, TGF-β inhibition is associated with increased inflammation of the lung (pneumonia). The combination of anti-TGF-β agents with inhibitors of inflammatory mediators (COX-2 inhibitors) results in optimal immunotherapy, which is comparable to chemotherapy and is partially additive with chemotherapy.[11]

3. Inhibition of IL-4 (D Lowrie)

Anti-IL-4 has a prolonged therapeutic effect in mice (BALB/c), even when administered late after infection.[12] IL-4 inhibits expansion of CD8+ CTL. The anti-IL-4 appears to mimic one effect of DNA vaccines, possibly by blocking stimulation of IL-4 receptors.

4. RUTI – fragmented *M. tuberculosis* delivered in liposomes (G Rook)

RUTI, devised by Dr PJ Cardona (Barcelona), is made from mechanically fragmented *M. tuberculosis* from which the lipid-rich supernatant is removed.[13] RUTI contains Hsp65. The therapeutic effect may be due to the strong induction of CD8+ IFN+ T cells. RUTI also induces a Th2 and large antibody response and may also induce TGF-β. It has no therapeutic effect in late progressive TB disease in the absence of chemotherapy.

6.1.2 Agents studied in humans

1. Human immunoglobulin (IVIg) (S Jolles)

The human blood product IVIg contains 95% of IgG subclass and is an immunomodulatory agent when used in high doses. It has been used in a wide range of immune and inflammatory disorders and is anti-infectious in a range of viral and bacterial infections. Its mechanism of action is only partially understood. One report suggests that sialylation of the N-linked biantennary sugar present on a glycosylation site on the Fc region of IgG leads to a ten-fold increase in anti-inflammatory potency.[14] High doses of IVIg administered to tuberculous mice resulted in a sustained significant reduction in bacterial counts, whether administered in the early or late stages (unpublished data, S Jolles). The protective effect of IVIg persists beyond its half life; its effect may be mediated through conventional T cell responses, cytokine production and effects on regulatory T cells rather than inhibition of mycobacterial growth within macrophages. No trials of high dose immunoglobulin have yet been conducted in patients with TB.

6.1.3 Agents studied in mice and humans

1. Killed *M. vaccae* (J Stanford, G Rook)

M. vaccae is a harmless saprophytic environmental mycobacterium found in untreated water and mud, it contains mycobacterial Hsp65. Most antigens of *M. vaccae* are cross-reactive with *M. tuberculosis*, Studies of *M. vaccae* have been conducted in mice, guinea pigs, badgers, cattle, rabbits, and dogs with eczema. Studies in humans have been carried out on healthy subjects and in patients with leprosy, TB, HIV infection, allergic dermatitis (children and adults), asthma, psoriasis, rheumatic diseases and cancer.

In mice, *M. vaccae* vaccination is associated with an increase in IFNγ-secreting cells and CD8 CTL that will kill TB-infected macrophages, down-regulation of IL-4 by regulatory T cells and possibly proinflammatory glycoforms of IgG.[15-17] *M. vaccae* has a therapeutic effect when administered after induction of a Th2 response, as occurs with TB, through the induction of antigen-specific Th2-suppressing regulatory T cells, which down-regulates the Th2 (IL-4) response, while promoting Th1 and CD8 CTL responses.[18,19] In mice, oral administration of *M. vaccae* is as effective as subcutaneous. (R Henandez-Pando and G Rook, unpublished data).[20] An oral formulation of *M. vaccae* is in development for humans. This will have lower costs, ease of administration and no risk of HIV transmission.

Amongst trials in other diseases, a number of small clinical trials have been conducted in TB patients with pulmonary disease using a single dose of *M. vaccae*. The effects differed by geographical location. The South African trial showed no effect. Trials in Argentina, India, Nigeria, Romania and Viet Nam showed benefits from faster bacteriological conversion, increases in weight and radiological resolution when given to TB patients receiving DOTS.[21] Three major efficacy trials using a single dose of *M. vaccae* have been conducted in Africa. The South African trial showed no effect.[22] The Ugandan trial was effective in achieving faster sputum clearance and radiological resolution.[23] The study in Zambia and Malawi showed a trend (p=0.06) towards a benefit in HIV-uninfected individuals and no effect in HIV-infected individuals.[24] Specific studies in HIV-infected persons have demonstrated safety, most cases required repeated injection for an effect to be observed. Currently a large trial of five doses of *M. vaccae* is running in Tanzania, investigating its potential value in the prevention of tuberculosis in HIV positive patients.

Dlugovitzky, Stanfords and colleagues have undertaken (published and unpublished) studies on TB patients receiving either single or triple doses of *M. vaccae* as well as chemotherapy. These have shown that the immunological response in humans is similar to that observed in mice: Serum IL-4, TGF-β and TNF-α all decrease and IFNγ and IL-2 are increase.[25] Cell studies showed a faster return towards normal values in respiratory bursts and CD11b expression on both polymorphs and monocytes in *M. vaccae* recipients, demonstrating a reduction in Th2 and increase in Th1 activity.

Multiple doses of *M. vaccae* have been evaluated in MDR-TB patients in a few small pilot studies. These suggested faster culture-conversion rates and radiological resolution and greater cure rates. Multiple doses were well-tolerated and could now be evaluated in good clinical practice trials in M(X)DR-TB. An oral formulation of *M. vaccae* is in development for humans. This may have lower costs, be easier to administer and avoid the risk of HIV transmission.

2. Dehydroepiandrosterone (DHEA), androstenediol (AED) and 16α-Bromoepiandrosterone (C Reading, J Frincke)

A synthetic derivative of DHEA, which is not readily metabolized into sex steroids, restores T helper cell Type I activity and accelerates bacterial clearance in tuberculous mice (BALB/c). An increased expression of TNF-α, IFNγ and iNOS and reduced expression of IL-4 was observed.[26] DHEA is neither synergistic nor additive when administered with M. vaccae in tuberculous mice.[15]

HE2000, a synthetic DHEA derivative (16α-Bromoepiandrosterone), has been evaluated in nine clinical trials for HIV, hepatitis B and malaria. This is generally safe with either buccal or subcutaneous routes of administration. The commonest adverse event with subcutaneous and intramuscular (IM) injection is a reaction at the injection site with local erythema, induration and pain. In HIV-infected treatment-naïve patients, HE2000 resulted in an increase in circulating IFNγ+ CD8+ T cells, an increase in CD8+ T cell responses against HIV peptides (gag) and a decrease in viral load.[27] In a randomized trial of HE2000 administered to 26 AIDS patients, at a time when antiretroviral therapy was not available, HE2000 was associated with a significant reduction in the incidence of TB and opportunistic infections. No trials of HE2000 have yet been conducted in patients with TB.[28]

3. Interferon

Interferon (IFN) γ is essential for antimycobacterial host defences. There have been five small trials of aerosolized or IM IFNγ or α. Although the first study, by Condos[29] showed an effect (sputum smears became negative and sputum CFU counts decreased), none of the other studies showed a long term microbiological benefit,[30-32] including the only placebo-controlled study that was stopped due to lack of effect.[33] A trend towards earlier sputum-smear conversion was reported recently among preliminary findings from a controlled trial in which patients with drug-sensitive TB were treated with DOTS alone or with added inhaled or systemic IFNγ.[34]

4. Interleukin-2

Interleukin-2 (IL-2) promotes T cell replication and is essential for cellular immune function and granuloma formation. In 1997, a small, unblinded study of two low-dose IL-2 regimens (daily or in 5-day "pulses") found that the daily regimen appeared to decrease sputum AFB counts.[35] Based on this preliminary observation, a randomized, double blind, placebo-controlled trial of the effect of IL-2 on sputum-culture conversion was conducted in 110 HIV-uninfected drug-sensitive Ugandan TB cases.[36] Contrary to expectations, the study found reduced clearance of viable *M. tuberculosis* sputum counts and delayed sputum culture-conversion in the IL-2 arm. This finding has been interpreted as indicating the potential for antagonism between a bacteriostatic granulomatous response and the bactericidal effects of chemotherapy (Wallis 2005).

5. *Mycobacterium w* (MW)

Mycobacterium w (MW) is an environmental species of uncertain taxonomy. An autoclaved preparation of this organism is licensed in India as an immunomodulator for intradermal injection in multibacillary leprosy. In mice it induces a T cell response that includes IFNγ and IL-2.[37] There is a report that a subcutaneous injection of this material in mice reduces the lethality of an extremely high intraveous challenge with M. tuberculosis (107 cfu) given four weeks later.[38] Mortality was assessed four weeks after challenge, and this model is not comparable with other work on TB immunotherapy in the literature. The mechanism of the effect seen is unclear. A single preliminary study in human tuberculosis compared time to sputum conversion in patients receiving conventional anti-TB therapy, with time to sputum conversion in patients receiving both anti-TB therapy and fortnightly intradermal injections of autoclaved MW.[39] More rapid conversion is suggested but no statistical analysis is provided, and the data are not listed in a format that allows such an analysis to be performed.[39] Further studies with MW in TB are planned.

6.2 Immunotherapy that increases chemotherapy access

M. tuberculosis adapts to conditions within granulomas by adopting a dormancy phenotype (with reduced replication and aerobic metabolism, and altered biosynthesis) against which most current TB drugs have reduced bactericidal activity. The strategies described below seek to restore these drugs to full effectiveness by increasing drug access and bacillary responsiveness. There are two potential concerns: a dependence on concomitant bactericidal therapy (uncertain in XDR-TB); and the risk that generalized immunosuppression may result in opportunistic superinfections.

6.2.1 Thalidomide analogues (G Kaplan)

Tumour necrosis factor alpha (TNF-α) is essential for granuloma formation and contributes to the activation of macrophages, which controls the growth of *M. tuberculosis*. Inhibiting TNF-α would modify the environment in which bacilli reside and might make the organism less likely to go into latency and more responsive to the bactericidal action of antibiotics.

This hypothesis was tested in mice (C57BL/6) infected with a low dose of TB (HN878). Mice in the control group were able to contain, but not kill, the infection. Administration of a TNF-α inhibitor (CC-3052, a Thalidomide analogue) alone from the time of infection resulted in partial inhibition of TNF-α. Treatment with isoniazid alone cleared the infection at a steady state but did not sterilize the lung. A combination of the TNF-α inhibitor and isoniazid resulted in faster clearance of bacteria. TNF-α inhibition resulted in reduced intracellular expression of TNF-α in splenocytes. Thus, immune modulation with TNF-α inhibitors accelerates bacillary killing by antibiotics and probably reduces the number of organisms that are latent and can be reactivated later. This strategy can be used to treat drug-resistant TB to improve the sterilizing action of antibiotics to which the organism is still sensitive. Inhibition of TNF-α also will contribute to clinical improvement by reducing the toxic effects of inflammatory mediators including TNF-α.

6.2.2 Etanercept (soluble TNF-a receptor) (R Wallis)

A phase I study examined the response to treatment in 16 subjects given adjunctive etanercept 25 mg subcutaneously twice-weekly for 8 doses, beginning on day 4 of standard TB treatment.[37] Sputum culture conversion occurred a median of 7 days earlier in the etanercept arm (p = 0.04). Etanercept was well-tolerated.

6.2.3 High dose prednisolone (R Wallis)

A substantially greater microbiological benefit was observed in a phase II study in which 189 subjects were treated with prednisolone (2.75 mg/kg/day) or placebo for the first month of standard TB chemotherapy.[41] This daily dose was based on phase I studies indicating that it had halved TB-stimulated TNF-α production ex vivo; no previous studies have examined the microbiological effects of doses of this magnitude. The median time to sputum culture conversion was one month earlier in prednisolone-treated patients (p = 0.001). This is the greatest effect on sputum culture conversion observed in any trial of adjunctive therapy in TB, whether chemotherapy or immunotherapy. There were no serious infectious complications; however, corticosteroid metabolic and cardiovascular effects were more common in prednisolone-treated subjects. Future studies may consider reducing these adverse events by delivering steroid therapy to the lung by inhalation, or by using a targeted anti-TNF-α therapy with greater anti-granuloma activity, such as infliximab.

6.3 Recommendations for therapeutic modulatory agents (working group 1)

The group supports immunomodulatory therapy as an adjunct to TB treatment. The consensus is that an optimized immune response will facilitate bacillary clearance and reduce immunopathology. Consequently agents that increase the protective immune response, or down-regulate pathways that are inappropriate or lead to immunopathology, are likely to be useful as adjunctive therapy in controlling TB. This position is based on observation of safety and efficacy data in mice and, for some compounds, human data.

The magnitude of the TB/XDR-TB crisis requires funding to be committed to fast-tracking product development of existing candidate immunotherapies. Immunomodulatory agents should be developed in addition to the efforts to find new antibiotics and chemotherapeutic agents, and should not be seen as competing. Immunomodulators aim to enhance or optimize the efficacy of chemotherapy.

The candidates chosen for review were those with published data that are either already available GMP, or expected to be available GMP, within a few months. However we realize that other strategies are in the early stages of investigation and would like to encourage further upstream innovation.

Two distinct types of immunomodulatory agent have been considered.

- Those that enhance protective immunity and/or down-regulate Th2 activity.
- Those that facilitate access of chemotherapeutic agents to the bacilli, by disrupting bacteriostatic pathways, macrophage function or granuloma integrity.

The characteristics of the immunotherapy agents are summarized in Table 1 and Table 2 and considered separately below.

6.3.1 Agents that enhance protective immunity and/or down-regulate Th2 activity

The agents were evaluated in relation to three possible scenarios.

- Adjunctive therapy with available drug therapies. It is envisaged that, where possible, this strategy will be used for initial evaluation.
- Therapy in the absence of available drugs. This may be necessary in certain XDR-TB patients.

- Immunotherapy targeted at HIV-infected individuals for prophylaxis of TB and other opportunistic infections (HE2000 *M. vaccae* is currently in such a trial).

Other possible uses of immunotherapy, such as shortening of treatment regimens, were not considered, although we acknowledge this potential.

It was noted that six of the agents – Hsp65 DNA vaccine, *M. vaccae*, anti-IL-4 (considered alongside data from IL-4 knockout in an animal model), blocking TGF-β, HE2000 and *Mycobacterium w* – shared certain properties in the animal models or in humans that might indicate a common final pathway. These reagents all led to falls in IL-4 and TGF-β and to increases in cytotoxic CD8+ cells. More work is required to prove that this is indeed a common final pathway, but it does suggest that similar biomarkers, assays and methods could be used for trials of all of these reagents.

1. Animal versus human studies

These reagents can be classified according to whether they have been studied in mice, humans, or both.

- Studies in mice only:
  - ◊ soluble Type III TGF-β receptor + COX2 inhibitor
  - ◊ Hsp65 DNA vaccine
  - ◊ anti-IL-4 (different antibodies tested in humans for other indications).
- Studies in humans, but not TB patients
  - ◊ IVIg
  - ◊ HE2000 (studies in HIV, but incidence of TB documented).
- TB studies in humans
  - ◊ *M. vaccae* (modified manufacturing process will necessitate repeat studies)
  - ◊ rh-IFNγ
  - ◊ *Mycobacterium w*.

2. Regulatory status

Reagents also can be classified according to their status in the regulatory approval process.

- Approved for other clinical indications; physicians can use off-label: IVIγ & rh-IFNγ.
- Proceeding through regulatory processes: *M. vaccae*, HE2000 & IL-4 inhibitor.
- Undergoing pre-clinical testing: Hsp65 DNA, soluble Type III TGF-β receptor + COX2 inhibitor.

However, with the exception of *M. vaccae*, none of the products has completed testing for safety in TB patients (some work with *M. vaccae* must be repeated because of manufacturing changes). We recommend that these products complete evaluation for safety and efficacy in TB patients so that they will be available for potential use in responding to the TB/XDR-TB crisis.

Although these molecules are likely to be tested initially in HIV-uninfected patients they should also be evaluated for safety and efficacy in HIV-infected TB patients.

6.3.2 Agents that facilitate access of chemotherapeutic agents to the bacilli, by disrupting bacteriostatic pathways, macrophage function or granuloma integrity

Inhibitors of inflammatory mediators act differently to those that enhance immune protection – they inhibit inflammation and may reduce bacteriostasis (prednisolone, etanercept).

Published experimental data indicate that these molecules are expected to facilitate the killing of TB by antibiotics or chemotherapeutic agents, and reduce immunopathology. However, safety evaluations will need to consider that they inhibit the protective immune response. Immune suppressives may compromise any residual protective immune response, thereby encouraging increased growth of the organisms. In the presence of effective anti-TB drugs this may facilitate clearance of the organism. However, in the absence of adequate therapeutic regimens (e.g. for XDR-TB) this is likely to have a detrimental effect so safety will be the emphasis of the evaluation.

6.3.3 General recommendations

TDR endorsement of the immunotherapeutic approach will facilitate fund-raising by companies and academics that have potential therapies. It will also be seen as a positive and creative response to the XDR-TB crisis. Moreover, immunotherapy might become a significant component of TDR's TB portfolio, in which it could play a unique role.

The Immunotherapy Working Group should continue to advise WHO on future development of existing and future candidates. It might be sensible for TDR and the individuals/organizations that have immunotherapeutics to move forward as a consortium, and to lobby important potential funding agencies as such. For instance, this report lists proposed clinical markers for the different products. The considerable overlap (e.g. decreases in IL-4 and TNF-α, increased IFNγ and T cell function), suggests that several of the agents might target common immunological pathways in humans. A TDR-coordinated consortium might facilitate efforts to coordinate and standardize the assays used in future studies of these immunomodulatory reagents.

Table 1. Characteristics and status of immunotherapy agents

	Preclinical toxicology completed	Phase I safety established	Phase I/II preliminary efficacy	GMP product availability	Initial therapeutic target	Therapeutic goal	Product potential
Enhancing protective immunity							
M. vaccae	Yes 1	Yes	Yes	Yes 1	+/- MDR, +/-HIV	Clinical improvement/ survival, STSC	Will be inexpensive, scaleable, available for production
Hsp65 DNA	Yes	No	None	Yes	MDR	STSC	Inexpensive, scaleable, available for production
TGF-b inhibitor + Cox 2 inhibitor	No	No	None	No	DS	STSC	Available, patented
HE 2000	Yes	Yes	Yes	Yes	HIV and or TB	Prophylaxis, STSC, survival	Inexpensive, scaleable, available for production
IL-4 antibody	Yes	Yes	None	Yes	DS	STSC	Expensive, scaleable, currently not available
IVIg	Yes	Yes	No	Yes	DS	STSC	Available, scaleable, expensive
Rh-IFNg	Yes	Yes	Yes	Yes	MDR	STSC	Available, scaleable, expensive
Increase chemotherapy access							
Etanercept	Yes	Yes	Yes	Yes	DS	STSC	Available, scaleable, expensive
Corticosteroids	Yes	Yes	Yes	Yes	DS	STSC	Available, scaleable, inexpensive

MDR = multidrug-resistant, DS = drug susceptible, STSC = shortening time to sputum clearance. 1 = Testing and production of new formulation of *M. vaccae* presently underway.

Table 1. Characteristics and status of immunotherapy agents (continued)

	Next step	Known toxicity	Dose	Schedules	Cold chain	Storage	Concerns and recommendations	Potential biomarkers
Enhancing protective immunity								
M. vaccae	Complete GMP production	Injection site reaction	1 mg	Multiple weekly or monthly	No	Long shelf-life	New formulations need step by step validation	Redution in IL4
Hsp65 DNA	IND preclinical	NK	NK	Multiple toxicology	No	Long	Funding-dependent transition to human studies	No human experience
TGF-B inhib + Cox 2 inhibitor	GLP preclinical toxicology	NK (cardiotoxicity for COX2 inhibitors)	NK	Multiple	Yes	NK	Requires regulatory pathway	No human experience
HE2000	Phase IIb	Injection site reaction	100 mg	Multiple 5 daily doses every 6 weeks	Ambient	> 2 years	Funding-dependent transition to phase II TB studies	Decreased IL-4, decreased TNF-α
IL-4 antibody	Efficacy in in primates	NK	NK	NK	Yes	NK	GSK buy-in required	Don't know, ask GSK
IVIγ	Phase IIa in TB patients	Known safety profile	2 g/kg	Single cycle over 2 or 3 days	Varies	>1 yr at +4 °C and 3 months	Funding for trials in TB patients	No TB patient experience
rh-IFNγ	Phase II in TB	Flu-like, myelosuppression	NK	Daily	Yes	Long	Awaiting result of ongoing phase II	IFNγ induced genes
Increase chemotherapy access								
Etanercept	Phase II in TB	Potential infection risk	50 mg	Weekly	Yes	Long	Funding for additional TB patients studies required	Reduced sputum IFNγ
Corticosteroid	Phase II in TB	Glucocorticoid toxicity	1-2 mg/kg	Daily	No	Long	Funding for additional TB patients studies required	Reduced sputum IFNγ and TNF-α

NK = not known.

7 Surrogate markers for MDR-TB immunotherapy *(R Wallis)*

The classic endpoints in studies of TB therapy are the proportion of subjects whose sputum fails to clear at the end of therapy (failures) plus the proportion with recurrent disease during the two years post-therapy (relapses). The urgency of the present situation demands acceleration of the pace of clinical development. Surrogate markers have become increasingly important to this objective. The most desirable of these measure early during treatment a parameter that accurately predicts later relapse risk, regardless of the type or mechanism of action of the therapy under investigation. Such markers would facilitate drug development by reducing study size and duration. They may help to reduce patient exposure to experimental therapies, thus reducing the risk of participation. In the post-licensing clinical setting, surrogate markers also may assist early identification of patients with anticipated poor clinical outcomes, so that their treatment may be modified appropriately.

Surrogate markers should:

- measure a parameter linked closely to disease pathogenesis
- predict long-term outcome in the absence of specific therapy
- show large changes early in the course of treatment
- capture a treatment's full effect on the disease process
- be independent of the type or mechanism of action of the treatment.

7.1 Surrogate markers using sputum culture

Most microbiological TB markers are based on the observation that successful treatment is accompanied by a progressive reduction in the number of viable bacilli in sputum and sputum cultures becoming negative during the first two to three months of treatment. The best-studied of these markers, and closest to validation, is sputum culture status after two months of treatment, based on a retrospective analysis of eight large controlled trials.[42, 43] Subsequent studies, including one retrospective analysis of outcomes in MDR-TB treatment, largely have confirmed this observation.[44, 46] The shortcomings are relatively large sample-size requirements, long duration of treatment, and poor performance characteristics in predicting relapse in individual patients (sensitivity=50%, positive predictive value=18%).

Two alternative approaches may help to address these shortcomings. The first increases the frequency of sputum cultures to biweekly or weekly; time to sputum culture conversion becoming the outcome measure. The main concern with this lies in the handling of data from cases in which a later sputum culture is positive with a small number of colonies. The second approach examines the rate of decline of colony counts in quantitative sputum cultures performed repeatedly during the first month of treatment.[47] Non-linear mixed effects (NLME) modelling of such data reveals two bacillary populations. The first, killed rapidly, corresponds to early bactericidal activity (EBA).[48] The second, killed more slowly, corresponds to sterilization.[49] NLME analysis appears to make the most efficient use of limited data by accounting for individual variation and missing samples, however, the optimal timing of specimen collection is yet to be determined. The main disadvantage of this approach is that a relatively large number of sputum samples must be processed, serially diluted, cultured and counted. This may unduly expose laboratory personnel to infection with MDR-TB, and may be difficult to increase in scale to validate as a predictor of relapse.

7.2 Other microbiological markers

At the 2005 World Congress on Lung Health, Goletti and Tomei presented a paper in which polymerase chain reaction was used to detect small fragments (67 bp) of IS6110 DNA in the urine of all 20 patients with newly diagnosed pulmonary TB, and in none of 10 controls. The fragments apparently arise due to apoptosis of infected macrophages. DNA was undetectable after two months of treatment. As a non-sputum marker, this may overcome many of the limitations of sputum sampling, reflecting total body TB burden. This early observation requires confirmation.

7.3 Immunological surrogate markers

Several immunological factors have been studied as possible surrogate markers of the response to TB therapy. These include the frequencies of IFNγ-producing ESAT-6 responsive T cells in the blood, and levels of IFNγ, TNF-α and other cytokines in the sputum. These factors decline with successful treatment but not in treatment failures. It is not yet known whether they can serve as indicators of relapse risk.

7.4 Recommendations for use of markers for TB treatment response (working group 2)

Presently there are no fully validated surrogate markers for treatment failure or relapse in TB. However, one marker – the proportion of subjects with sputum culture conversion to negative after eight weeks of therapy – is considered close to validation, and others are promising candidates. The rapid development of adjunctive immunotherapies for TB will require the development and use of both microbial and immunological surrogate markers.

7.4.1 Immunological markers (early phase II studies)

The immunological markers under consideration here will be linked closely to the mechanism of action of immunological interventions. For this reason, they will be particularly useful in the early clinical development of new immune-based therapies – to detect a proximate effect of the intervention, serve as an initial guide to dose selection, and to monitor the duration of its effect.

Two approaches have been proposed for adjunctive TB immunotherapy: either to enhance factors associated with protection, or to reduce factors associated with immunopathology and persistence. Markers indicating proximate immunological effects of candidate immunotherapies will be invaluable in the conduct of early phase clinical trials. These markers most likely will be selected for specific studies based on the mechanism of action of the therapy under evaluation. Candidate markers include TB-specific CD4 and CD8 responses (by ELISPOT and flow cytometry), and cytokines associated with protection (IFNγ and IL-4 splice variant), risk (IL-4, TGF-β, IL-10), or inflammation and pathology (TNF-α, IL-6). Methods for accurate and reproducible measurement of these parameters are in different stages of standardization. Some are available as highly reproducible commercial assays. Others have, or are being, standardized by research consortia such as Biomarkers for TB (www.biomarkers-for-tb.net), supported by the Bill and Melinda Gates Foundation, and the NIH-funded TB Research Unit (http://www.case.edu/affil/tbru/).

7.4.2 Microbiological markers (late phase II and phase III studies)

Microbiological markers will comprise the main indicators of successful TB treatment in later stage studies. Although sputum culture status after two months of treatment currently is the best-validated marker, it falls short of expectations in its sensitivity and positive predictive value for relapse. It also has the disadvantages of requiring a relatively long treatment period (two months) and a relatively large sample size. Alternative markers include the closely related marker – time to sputum culture con-

version – and the rate of decline of serial sputum colony counts (SSCC) analysed by nonlinear mixed-effects modelling. Although SSCC data are limited, recent studies indicate that it can substantially reduce sample size requirements, and shorten the required duration of treatment to one month.

7.5 Future directions and research needs

The studies cited above indicate potentially complex interactions between immunotherapy and chemotherapy that may be treatment-specific and mechanism based. It is unlikely that a single surrogate marker will be sufficient to meet the needs of accelerated research. At a minimum, separate immunological markers will be required to assess proximate effects of immunotherapy on the host. Additional ex vivo experiments such as whole blood culture will be helpful for examining specific drug-drug and drug-immune interactions and to examine the dose-response relationship in the early phase evaluation of candidate regimens.

Additional research will be required to standardize the use of automated methods for quantitative sputum microbiology such as MGIT or Bactec. Time to detection of growth in these systems is inversely related to log inoculum size.[50, 51] This parameter increases progressively during treatment and may be a marker for failure or relapse,[52, 54] but additional research is needed. The use of these automated, sealed-culture systems may facilitate the validation of quantitative sputum microbiology as a predictor of relapse in large trials and may reduce potential risk to laboratory personnel in MDR-TB studies.

Lastly, the use of other types of specimens, such as urine (transrenal) TB DNA, may have reduced sample preparation requirements; reduced infection risk; and superior representation of the total body infection burden.

Initial evaluation of these and other potential markers in the early stages of development will be by comparison with two-month sputum culture conversion. Ultimately all tests must be validated by assessing their ability to predict failure and relapse in longitudinal cohort studies of 18-months duration. We strongly encourage WHO TDR to facilitate and promote efforts to harmonize and standardize the development of these markers.

8 Designing studies to evaluate TB immunotherapy: challenges and opportunities *(J Levin)*

In this discussion of potential issues surrounding the design of TB immunotherapy clinical trials it is assumed that a particular agent has shown promise in animal studies (i.e. shows some evidence of either an effect against *M. tuberculosis* or of a favourable immune response) and that animal safety studies have also been carried out. Further it is assumed that Phase I studies in human subjects have confirmed safety and some evidence of an immunological response.

Initially, Phase II studies need to be designed to determine dosing regimens that are safe and tolerable, most likely to be efficacious and to screen between alternative agents or a combination of agents. Thereafter, Phase III studies should be conducted to establish the efficacy of a proposed regimen for treating drug-resistant TB or shortening the duration of treatment for drug-susceptible TB.

8.1 Outcome measures

A clinical trial determines outcome measures or endpoints in each subject. The aggregate of these is required to evaluate the trial objectives. Generally, "hard" endpoints that require no subjectivity are preferred – such as all cause mortality. However, this requires large sample sizes and long follow-up times as the number of expected events is small. Some trials use a composite endpoint in order to increase the number of events, however, this requires clear guidelines on determining endpoints and a committee to adjudicate them. A composite endpoint of "death or TB relapse" could be used in a TB/HIV trial, but this would require reinfection to be distinguished from relapse through the use of TB strain typing (RFLPs). One criticism of composite outcomes is that while groups may agree on the overall composite outcome, they may differ on a particular component. If this is an important component (such as death), the trial may miss an important difference between groups.

8.2 Surrogate markers

Surrogate markers have been used to reduce the duration of a trial and the required sample size. A surrogate endpoint is chosen in place of the biologically definitive or clinically most meaningful endpoint as an impression of efficacy may be obtained sooner or more cheaply. Prentice gave a definition of the ideal requirement for a surrogate endpoint.[55] "A surrogate endpoint is a response variable for which a test of the null hypothesis of no relationship to the treatment groups... is also a valid test of the corresponding null hypothesis based on the true endpoint". While this ideal may be unattainable in practice, it provides a target for choosing a surrogate endpoint. Notably, a measurement that is significantly associated with the true outcome will not be a useful surrogate unless it also reflects the effects of treatment on the definitive outcome.[56, 61] The main difficulty with surrogate endpoints is whether an association between treatment arm and surrogate marker can be used to deduce an association with the definitive outcome (validity). An example of this lack of validity occurred in trials in cardiovascular disease: two major antiarrhythmic drugs (encanaide and flecainide) reduced arrhythmia but caused a more than three-fold increase in overall mortality.[62] Any surrogate marker that is to be used in TB immunotherapy trials should be subject to an ongoing process of validation that continues beyond any trial, and combines data from a number of trials.

A number of surrogate markers could be used in TB trials (including trials of immunotherapeutic agents). Phase IIA safety and dose-ranging studies could use appropriate immunological markers; Phase IIB tests of concept studies might use bacteriological markers such as SSCC or two-month culture conversion, which is less sensitive. Phase III efficacy trials probably would still need to use a conventional outcome such as cure. It would be useful to collect data on a range of potential surrogate markers (both immunological and bacteriological) in order to help to validate these for future use.

8.3 Superiority versus non-inferiority studies

Most Phase III clinical trials are carried out as superiority studies i.e. designed to show that the new treatment is more efficacious than the standard or reference treatment. However, a treatment which is no better on average than the standard treatment may still be useful, for example if it has a better safety profile. A non-inferiority trial aims to demonstrate that the test product is not worse than the comparator by more than a specified small amount, known as the non-inferiority margin or delta. A margin of non-inferiority is specified in the protocol and non-inferiority demonstrated if the two-sided 95% confidence interval for the true difference between the two drugs is entirely on the positive side of the non-inferiority margin. This requires pre-specification of the non-inferiority margin and must be justified on both clinical and statistical grounds.

"Gamma" – the true difference between standard and new treatments – also must be considered when determining sample size for a non-inferiority study. This concept can be demonstrated by a simple numerical example. If a standard six-month regimen has a two-year relapse rate of 5%, a shortened regimen should have a relapse rate of 10% at most. Delta, the maximum tolerable difference, is 5%. If the true relapse rate on the new treatment was 7% then gamma, the true amount by which the new treatment is worse than the standard, would be 7%-5% = 2%.

It should be noted that non-inferiority studies generally require far larger sample sizes than superiority studies. Fewer subjects would be required to demonstrate that an immunotherapeutic intervention improves the cure rate in drug-resistant TB (a superiority study) than to show that an immunotherapeutic agent added to a standard regimen allows shorter treatment (say four months) for drug-sensitive TB (a non-inferiority trial). If sample sizes are planned with over-optimism about the effect of an intervention (e.g. hoping that an immunotherapeutic agent will halve the failure rate) the study will be under-powered to detect more modest, but still clinically relevant, effects (e.g. 30% reduction in the failure rate).

8.4 Recommendations for study designs to evaluate immunotherapies for TB (working group 3)

In the light of the urgency for testing new agents that could be effective in treating drug-resistant TB (including XDR-TB) it is important to design trials as part of a holistic product development programme, taking account of all phases of clinical development. The working group discussed methodological constraints and made recommendations for study designs to evaluate the role of immunotherapy in improving the bacillary clearance for TB and M(X)DR-TB and the criteria for selecting clinical trial sites for M(X)DR-TB immunotherapy. Use of adaptive trial designs should also be considered, but is not discussed further in this report.

8.4.1 Drug susceptible and resistant TB

In early efficacy trials it is preferable to have uniform patient populations. However, M(X)DR-TB patients are heterogeneous and thus, initially (perhaps as part of Phase II safety studies), studies could be carried out in drug-sensitive patients. Microbiological surrogate markers could be used to provide evidence of the efficacy of immunotherapy as an adjunct to conventional anti-TB chemotherapy. Microbiological markers (such as two-month culture conversion and SSCC) or time to culture positivity using MGIT could be used. Such studies also could be used to assess the predictive value of the appropriate immunological markers, although this would increase the cost.

Subsequent studies could be carried out in drug-resistant patients, although trials should use MDR- and XDR-TB patients as it might be difficult to recruit only the latter. One challenge is the early diagnosis of drug resistance, so it was suggested that trials could be done in patients defined as early treatment failures according to national TB control programme guidelines. If this approach is followed, it is important that there is clear definition of an early treatment failure for the purposes of any given trial.

This case would require resistance testing for first-line drugs (particularly rifampicin and isoniazid) and for second-line drugs if rifampicin-resistant (particularly fluoroquinolones and the injectables). Thus trial sites will need the capacity to carry out drug-susceptibility testing for both first- and second-line drugs using standardized laboratory methods for determining drug resistance. It would be prudent to carry out at least the initial studies in a small number of sites, preferably one, to ensure homogeneity.

It is important to emphasize that immunotherapy is adjunctive to treatment in all studies (i.e. whether in drug-sensitive or drug-resistant patients). Patients should receive the standard of care recommended in treatment guidelines and carried out in collaboration with the national TB control programme. For drug-resistant TB this requires countries in which trials are undertaken to have access to second-line anti-TB drugs through the Green Light Committee. In addition, there needs to be consideration of which regimen to use in the treatment of M(X)DR-TB i.e. either fixed or individualized approach. Any trial must consider who will provide additional drugs (including second-line anti-TB drugs where these are not provided by the Green Light Committee; ART for HIV/TB co-infected patients). In many cases this would be the responsibility of the study sponsor.

It would also be useful to stratify MDR-TB patients according to whether or not they have resistance to fluoroquinolones in addition to isoniazid and rifampicin. Initially, separate studies should be carried out in HIV-uninfected and -infected TB patients.

8.4.2 Studies in HIV-uninfected TB patients

Patients can be randomized on failure of standard therapy (clearly defined and standardized in multi-centre trials). Subjects will be randomized to either standard of care (including changing the regimen for MDR-TB) or standard of care plus immunotherapy.

The primary outcome measure will be time (in months) to bacteriological clearance (month of first negative culture with no subsequent positive cultures). An additional microbiological endpoint such as SSCC or time to MGIT culture positivity could be measured as part of an ongoing exercise to validate these as surrogate measures. Similarly, the immunological markers discussed above (dependent on the type of immunomodulator) could be measured as part of a validation exercise. An additional outcome measure will be culture status two years from onset of treatment, validating the time to clearance and any other surrogate microbiological markers. Inclusion/ exclusion criteria will be modified according to the product under investigation. In particular, immunosuppressants require suitable precautions.

8.4.3 Trials in HIV-infected TB patients

These trials could be carried out either after the first studies in HIV-uninfected TB patients or, given the urgency of the situation of drug-resistant TB, in parallel with the studies in HIV-uninfected TB patients. Patients should be stratified on the basis of CD4 counts (e.g. $<$ or $\geq$ 250 cells/ml^{-3}). Patients in the low CD4 stratum should be given ART according to local treatment guidelines. The study should be powered sufficiently to detect the effect of immunotherapy separately in each stratum. Other aspects of the study design in HIV-uninfected TB patients would apply. Inclusion criteria should be modified to take account of precautions necessary for severely immunocompromised patients. These are likely to be multi-centre trials.

9 Conclusions

Dr Mario Raviglione, Director of the Stop TB Partnership, has set research and development of new interventions for the management and treatment of M(X)DR-TB as a top priority. The establishment of close collaboration between academics and all stakeholders would consolidate a global research movement.

The evidence reviewed in this document suggests that immunomodulators have the potential to improve the outcome of all TB including M(X)DR-TB. However, none of these agents have proof of efficacy for this use, although some are registered for other indications. We recommend that development and evaluation of potential agents for use in M(X)DR-TB are accelerated as fast as possible: an appropriate agency, such as the Stop TB Partnership, could coordinate fund-raising and facilitate dialogue with regulatory authorities.

Potential immunomodulatory agents should have phase II safety studies completed as soon as possible in TB patient populations that are accessible and homogeneous. Studies should move as fast as possible into M(X)DR-TB. Sites with large numbers of these patients, a secure supply of second-line drugs, and clinical trials and laboratory capacity should be identified and developed as potential study sites. The Global Alliance report on clinical TB trial sites may be useful for this purpose.

Although we recommend fast tracking of immune modulatory agents for treatment of M(X)DR-TB, these studies should be carried out in a rigorous manner to obtain scientifically and clinically meaningful results with particular regard to the safety issues of introducing immunomodulation in severely ill patients with compromised immune systems.

Appendix 1. Agenda

Expert consultation to evaluate the potential roles of immunotherapeutic interventions for TB in TB and HIV high-burden settings

January 2007

AGENDA

DAY 1

Time	Item	Name
9.00-9.10	Welcome and introductory remarks	R Ridley
9.10-9.20	Chairperson's opening comments	G Kaplan
9.20-9.30	Objectives and proposed outcomes of the meeting	P Onyebujoh
9.30-9.50	Global TB control in 2007: achievements, challenges, and the need for better tools	D Maher
9.50-10.10	Update on the activities of WHO Taskforce on XDR-TB	P Nunn
10.10-10.30	Discussion	
10.30-11.00	Coffee break	
11.00-11.20	Update on the activities of Vaccine Working Group: current candidates and developmental plans.	U Fruth
11.20-11.40	Immunomodulation for TB in high-burden settings for TB and HIV	G Rook
11.40-12.00	Discussions	
	Lunch break	
14.00-14.15	Development and potential use of biomarkers and/surrogate markers in TB	R Wallis
14.15-14.35	Introduction of Working Group: objectives and expected outcomes	Meeting rapporteur
14.35-16.30	Working Groups I, II, III	Rooms: to be assigned
16.30-16.45	**Coffee break**	
16.45-17.00	Summary of WG activities	WGs
	Closure 1st day	

DAY 2

Time	Item	Name
9.00-9.15	Objectives and outcomes for day 2	G Kaplan
9.15-9.35	New insight into the immunopathology of TB: prospects for improved interventions	Douglas Lowrie
9.35-10.00	Designing studies exploring TB immunotherapy: challenges and opportunities	Jonathan Levin
10.00-10.30	Discussion	
10.30-10.45	**Coffee break**	
10.45-12.30	Working Groups: continued	WGs
12.30-14.00	**Lunch break**	
14.00-15.30	Working Groups: continued	WGs
15.30-16.00	**Coffee break**	
16.00-16.20	Plenary report back Working Group I	WG rapporteur
16.20-16.40	Plenary report back Working Group II	WG rapporteur
16.40-17.00	Plenary report back Working Group III	WG rapporteur
17.00-17.30	Discussion	
17.30-18.00	Chairperson's review and summation of Day II activities	
	Closure 2nd day	

DAY 3

Time	Item	Name
09.00-09.15	Objectives and outcomes for Day 3	SWG Chairperson/ meeting rapporteur
09.15-10.15	Finalization of WG recommendations	
	Coffee break	
11.00-12.30	Finalization of WG recommendations	
12.30-14.00	**Lunch break and meeting closure**	
14.00-15.30	Meeting with Chairperson, WG chairpersons, main rapporteur and writer	
15.30-16.00	**Coffee break**	
16.00-17.00	Final discussion	
	Closure 3rd day	

Appendix 2. List of participants

Immunotherapy meeting

Geneva, SWITZERLAND, 29 - 31 January 2007

Temporary advisers

Dr Abraham ASEFFA
Deputy Director
Armauer Hansen Research Institute
Jimma Road
ALERT Compound
PO Box 1005
Addis Ababa, ETHIOPIA
Telephone: +251 113211334
Fax: +251 0113211563
E-mail: aseffaa2@yahoo.com

Dr Georges BAHR
Dean
Faculty of Sciences
Professor of Immunology and Virology
Faculty of Medicine, University of Balamand
PO Box 100
Tripoli, LEBANON
Telephone: +961 3370780
E-mail: georges.bahr@balamand.edu.lb

Dr Laura ROSA BRUNET
SR Pharma plc
26th Floor
Centre Point
103 New Oxford Street
London WC1A 1DD, UK
Telephone: +44 2073071625
E-mail: l.rosabrunet@srpharma.com

Professor Gavin CHURCHYARD
Chief Executive Officer
Aurum Institute for Health Research
PO Box 61587
Marshalltown 2107, SOUTH AFRICA
Telephone: +27 1163872604
Fax: +27 116382179
E-mail: gchurch@mjvn.co.za

Dr Jim FRINCKE
Chief Scientific Officer
Hollis-Eden Pharmaceuticals
4435 Eastgate Mall, Suite 400
San Diego, CA 92121, USA
Telephone: +1 8585879333
Fax: +1 8585586470
E-mail: jfrincke@holliseden.com

Dr Rogelio HERNANDEZ PANDO
Department of Pathology
National Institute of Medical Science and Nutrition
Salvador Zubiran
Vasco de Quiroga, No-15 14000
Tlalpan, MEXICO
Telephone: +52 5554853491
Fax: +52 5556551076
E-mail: rhdezpando@hotmail.com

Dr Stephen JOLLES
Department of Medical Microbiology
Cardiff University
Health Park
Cardiff CF14 4XN, UK
Telephone: +44 2920745814
Fax: +44 207488383
E-mail: jollessr@cardiff.ac.uk

Professor Gilla KAPLAN
The Public Health Research Institute
International Center for Public Heath (ICPH)
225 Warren Street
Newark,
NJ 07103-3535, USA
Telephone: +1 9738543220
Fax: +1 973854 3101
E-mail: kaplan@phri.org

Dr Jonathan LEVIN
MRC/UVRI Uganda Research Unit on AIDS
c/o Uganda Virus Research Institute
PO Box 49 - Entebbe, Uganda
Telephone: +27 0219380911
Fax: +256 41321137
E-mail: jonathan.levin@mrcuganda.org

Dr Douglas B. LOWRIE
Department of Medical Microbiology
Cardiff University
Health Park
Cardiff CF14 4XN, UK
Telephone: +44 2920744609
E-mail: lowried@cardiff.ac.uk

Dr Mohammed-Reza MASJEDI
Deputy Director
National Research Institute of Tuberculosis and Lung Disease
Shaheed Bahonar Ave.
Darband Tehran 19556, IRAN (ISLAMIC REP.)
Telephone: +98 2120109991
Fax: +98 2120109484
E-mail: mrmasjedi@nritld.ac.ir

Dr Carol NACY
Chief Executive Officer
Sequella Inc. - 9610 Medical Center Drive
Suite 200 Rockville
MD 20850, USA
Telephone: + 1 3017627776
Fax: +1 3017627778
E-mail: carolnacy@sequella.com

Dr Joseph OLOBO
Associate Professor
Department of Microbiology
Makerere University Medical School
PO Box 7072
Kampala, UGANDA
Telephone: +256 41531126
Mobile:+256 272613620
Fax: +256 41531126
E-mail: jolobo@med.mak.ac.ug

Dr Shreemanta K. PARIDA
Max-Planck Institute for Infection Biology
Charitéplatz 1
10117 Berlin, GERMANY
Telephone +49 3028460504
Fax: +49 3028460503
E-mail: parida@mpiib-berlin.mpg.de

Dr Christopher READING
Executive Vice President, Scientific Development
Hollis-Eden Pharmaceuticals
4435 Eastgate Mall, Suite 400
San Diego, CA 92121, USA
Telephone: +1 8585879333
Fax: +1 8585586470
E-mail: creading@holliseden.com

Dr Elisabeth Pereira SAMPAIO
Fundaçao Oswaldo Cruz
Departamento de Hanseniase
Av. Brasil - 4365 - Manguinhos
21045-900 Rio de Janeiro, BRAZIL
Fax: + 55 2122709997
E-mail: esampaio@gene.dbbm.fiocruz.br

Professor Graham ROOK
Centre for Infectious Diseases & International Health
Windeyer Institute of Medical Sciences
Royal Free and University College Medical School
46 Cleveland Street
London W1T 4JF, UK
Telephone: +44 2076799434
Fax: +44 2076799434
E-mail: g.rook@ucl.ac.uk

Professor John STANFORD
Former Head, Department of Medical Microbiology
Windeyer Institute of Medical Sciences
Royal Free and University College Medical School
46 Cleveland Street
London W1T 4JF, UK
Telephone: +44.1892730298
E-mail: johnls@dircon.co.uk

Ms Cynthia STANFORD
SNR
c/o Prof John STANFORD
Telephone: +44 1892730298

Dr Robert WALLIS
Medical Director
PPD
213 N St. NW, Suite A
Washington DC 20005, USA
Telephone: +1 2023604784
Fax: +1 9196540640
E-mail: robert.wallis@columbia.ppdi.com

Dr Gerhard WALZL
Molecular Biology and Human Genetics
Department of Health Sciences
Faculty of Health Sciences
University of Stellenbosch
FISAN Building 4th Floor
Francie Van zyil Avenue, Tygerberg 7505, SOUTH AFRICA
Telephone: +27 218089111
E-mail: GWALZL@sun.ac.za

Professor Ali ZUMLA
Centre for Infectious Diseases and International Health
University College London
Windeyer Institute of Medical Sciences
46 Cleveland Street
London W1P 6DB, UK
Telephone: +44 2076799311
Fax: +44 2076799311
E-mail: a.zumla@ucl.ac.uk

WHO Headquarters

Dr Robert G. RIDLEY
Director TDR
Telephone: +41 22 79 13802/13906
E-mail: ridleyr@who.int

Dr Mario RAVIGLIONE
Director STB
Telephone: +41 22 79 12663/13986
E-mail: raviglionem@who.int

Dr Jane F. KENGEYA-KAYONDO
Coordinator IRM
Telephone: 13737/14453
E-mail: kengeyakayondo@who.int

Dr Janis Karlin LAZDINS-HELDS
Coordinator PDE
Telephone: +41 22 79 13818/13738
E-mail: lazdinsj@who.int

Dr Ayoade ODUOLA
Coordinator SDR
Telephone: +41 22 79 13212/13789
E-mail: oduolaa@who.int

Dr Fabio ZICKER
Coordinator RCS
Telephone: +41 22 79 13805/13806
E-mail: zickerf@who.int

Dr Ulrich Josef FRUTH
Scientist
Telephone: +41 22 79 12678/13449
E-mail: fruthu@who.int

Dr Hashim GHALIB
Scientist
Telephone: +41 22 79 11270
E-mail: ghalibh@who.int

Dr Dermot Paul MAHER
Medical Officer
Telephone: +41 22 79 12655/11522
E-mail: maherd@who.int

Dr Nina MATTOCK
Technical Officer
(now retired)

Dr Paul P NUNN
Coordinator THD
Telephone: +41 22 79 12963/13544
E-mail: nunnp@who.int

Dr Piero Luigi OLLIARO
Scientist
Telephone: +41 22 79 13734/12729
E-mail: olliarop@who.int

Dr Philip Chukwuka ONYEBUJOH
Medical Officer
Telephone: +41 22 79 14478/12815
E-mail: onyebujohp@who.int

Dr Rosanna Wai-Wan PEELING
Medical Officer
Telephone: +41 22 79 13742/12261
E-mail: peelingr@who.int

Mr Andrew Robert RAMSAY
Scientist
Telephone: +41 22 79 11545/12261
E-mail: ramsaya@who.int

Dr Fabio SCANO
Telephone: +41 22 79 12858
Medical Officer
E-mail: scanof@who.int

Dr George SCHMID
Medical Officer
Telephone: +41 22 79 14834
E-mail: schmidg@who.int

Dr Mahnaz VAHEDI
Medical Officer
Telephone: +41 22 79 11065/12815
E-mail: vahedim@who.int

Dr Marco VITORIA
Medical Officer
Telephone: 11949
E-mail: vitoriam@who.int

Dr Brian Gérard WILLIAMS
Scientist
Telephone: +41 22 79 14680/14566
E-mail: williamsbg@who.int

REFERENCES

(1) Extensively drug-resistant tuberculosis (XDR-TB): recommendations for prevention and control. *Wkly Epidemiol Rec* 2006;81(45):430-2.

(2) Gandhi NR, Moll A, Sturm AW, Pawinski R, Govender T, Lalloo U, et al. Extensively drug-resistant tuberculosis as a cause of death in patients co-infected with tuberculosis and HIV in a rural area of South Africa. *Lancet* 2006;368(9547):1575-80.

(3) Lancet Editorial. XDR-TB--a global threat. *Lancet* 2006;368(9540):964.

(4) Raviglione MC, Smith IM. XDR tuberculosis – implications for global public health. *N Engl J Med* 2007;356(7):656-9.

(5) Rook GAW, Lowrie DB, Hernandez-Pando R. *Immunotherapeutics for tuberculosis in experimental animals: is there a common pathway activated by effective protocols?* Discussion document for Expert Consultation on TB Immunotherapy WHO-TDR 29-31 January 2007.

(6) Rook GAW, Lowrie DB, Hernandez-Pando R. Immunotherapeutics for tuberculosis in experimental animals; is there a common pathway activated by effective protocols? *J Infect Dis* 2007. In press.

(7) Lowrie DB, Tascon RE, Bonato VL, Lima VM, Faccioli LH, Stavropoulos E, et al. Therapy of tuberculosis in mice by DNA vaccination. *Nature* 1999;400(6741):269-71.

(8) Silva CL, Bonato VL, Coelho-Castelo AA, De Souza AO, Santos SA, Lima KM, et al. Immunotherapy with plasmid DNA encoding mycobacterial hsp65 in association with chemotherapy is a more rapid and efficient form of treatment for tuberculosis in mice. *Gene Ther* 2005;12(3):281-7.

(9) Nuermberger E, Tyagi S, Williams KN, Rosenthal I, Bishai WR, Grosset JH. Rifapentine, moxifloxacin, or DNA vaccine improves treatment of latent tuberculosis in a mouse model. *Am J Respir Crit Care Med* 2005;172(11):1452-6.

(10) Trinchieri G. Immunobiology of interleukin-12. *Immunol Res* 1998;17(1-2):269-78.

(11) Hernandez-Pando R, Orozco-Esteves H, Maldonado HA, Aguilar-Leon D, Vilchis-Landeros MM, Mata-Espinosa DA, et al. A combination of a transforming growth factor-beta antagonist and an inhibitor of cyclooxygenase is an effective treatment for murine pulmonary tuberculosis. *Clin Exp Immunol* 2006;144(2):264-72.

(12) Lowrie, D. B.. Potential of immunotherapy revealed in mice. Proceedings of 6th International Conference on Pathogenesis and Mycobacterial Infections, June 30 to July 3. 2005, Stockholm, Sweden.

(13) Cardona PJ. RUTI: a new chance to shorten the treatment of latent tuberculosis infection. *Tuberculosis (Edinb)* 2006 86(3-4):273-89.

(14) Kaneko Y, Nimmerjahn F, Ravetch JV. Anti-inflammatory activity of immunoglobulin G resulting from Fc sialylation. *Science* 2006;313(5787):670-3.

(15) Hernandez-Pando R, Pavon L, Orozco EH, Rangel J, Rook GA. Interactions between hormone-mediated and vaccine-mediated immunotherapy for pulmonary tuberculosis in BALB/c mice. *Immunology* 2000;100(3):391-8.

(16) Skinner MA, Prestidge R, Yuan S, Strabala TJ, Tan PL. The ability of heat-killed *Mycobacterium vaccae* to stimulate a cytotoxic T cell response to an unrelated protein is associated with a 65 kilodalton heat-shock protein. *Immunology* 2001;102(2):225-33.

(17) Skinner MA, Yuan S, Prestidge R, Chuk D, Watson JD, Tan PL. Immunization with heat-killed *Mycobacterium vaccae* stimulates CD8+ cytotoxic T cells specific for macrophages infected with *Mycobacterium tuberculosis*. *Infect Immun* 1997;65(11):4525-30.

(18) Zuany-Amorim C, Manlius C, Trifilieff A, Brunet LR, Rook G, Bowen G, et al. Long-term protective and antigen-specific effect of heat-killed *Mycobacterium vaccae* in a murine model of allergic pulmonary inflammation. *J Immunol* 2002;169(3):1492-9.

(19) Zuany-Amorim C, Sawicka E, Manlius C, Le MA, Brunet LR, Kemeny DM, et al. Suppression of airway eosinophilia by killed *Mycobacterium vaccae*-induced allergen-specific regulatory T-cells. *Nat Med* 2002;8(6):625-9.

(20) Hunt JR, Martinelli R, Adams VC, Rook GA, Brunet LR. Intragastric administration of *Mycobacterium vaccae* inhibits severe pulmonary allergic inflammation in a mouse model. *Clin Exp Allergy* 2005;35(5):685-90.

(21) Stanford J, Stanford C, Grange J. Immunotherapy with *Mycobacterium vaccae* in the treatment of tuberculosis. *Front Biosci* 2004;9:1701-19.

(22) Durban Immunotherapy Trial Group.Immunotherapy with *Mycobacterium vaccae* in patients with newly diagnosed pulmonary tuberculosis: a randomised controlled trial. *Lancet* 1999; 354(9173):116-9.

(23) Johnson JL, Kamya RM, Okwera A, Loughlin AM, Nyole S, Hom DL, et al. Randomized controlled trial of *Mycobacterium vaccae* immunotherapy in non-human immunodeficiency virus-infected Ugandan adults with newly diagnosed pulmonary tuberculosis. The Uganda-Case Western Reserve University Research Collaboration. *J Infect Dis* 2000;181(4):1304-12.

(24) Mwinga A, Nunn A, Ngwira B, Chintu C, Warndorff D, Fine P, et al. *Mycobacterium vaccae* (SRL172) immunotherapy as an adjunct to standard antituberculosis treatment in HIV-infected adults with pulmonary tuberculosis: a randomised placebo-controlled trial. *Lancet* 2002;360(9339):1050-5.

(25) Dlugovitzky D, Fiorenza G, Farroni M, Bogue C, Stanford C, Stanford J. Immunological consequences of three doses of heat-killed *Mycobacterium vaccae* in the immunotherapy of tuberculosis. *Respir Med* 2006;100(6):1079-87.

(26) Hernandez-Pando R, De La Luz SM, Orozco H, Arriaga K, Pavon L, Al-Nakhli SA, et al. The effects of androstenediol and dehydroepiandrosterone on the course and cytokine profile of tuberculosis in BALB/c mice. *Immunology* 1998;95(2):234-41.

(27) Reading C, Dowding C, Schramm B, Garsd A, Onizuka-Handa N, Stickney D, et al. Improvement in immune parameters and human immunodeficiency virus-1 viral response in individuals treated with 16alpha-bromoepiandrosterone (HE2000). *Clin Microbiol Infect* 2006;12(11):1082-8.

(28) Stickney D, Nuermberger E, Garsd A, Dettoni V, Frincke J. The effect of HE2000, an immune modulator, on the incidence of tuberculosis and other opportunistic infections in AIDS patients. *Antimicrobial Agents and Chemotherapy*. 2007; 51: 2639-2641

(29) Condos R, Rom WN, Schluger NW. Treatment of multidrug-resistant pulmonary tuberculosis with interferon-y via aerosol. *Lancet* 1997;349(9064):1513-5.

(30) Giosue S, Casarini M, Ameglio F, Zangrilli P, Palla M, Altieri AM, et al. Aerosolized interferon-alpha treatment in patients with multidrug-resistant pulmonary tuberculosis. *European Cytokine Network* 2000;11(1):99-104.

(31) Suarez-Mendez R, Garcia-Garcia I, Fernandez-Olivera N, Valdes-Quintana M, Milanes-Virelles M, Carbonell D, et al. Adjuvant interferon gamma in patients with drug-resistant pulmonary tuberculosis: a pilot study. *BMC Infectious Diseases* 2004;4(1):44.

(32) Koh WJ, Kwon OJ, Suh GY, Chung MP, Kim H, Lee NY, et al. Six-month therapy with aerosolized interferon-gamma for refractory multidrug-resistant pulmonary tuberculosis. *Journal of Korean Medical Science* 2004;19(2):167-71.

(33) Business Wire. InterMune Enrolls First Patient in Phase III Trial in Multidrug-Resistant Tuberculosis, August 1, 2000 Business/Health Editors BURLINGAME, Calif.--(BW HealthWire)--Aug. 1, 2000 (http://findarticles.com/p/articles/mi_m0EIN/is_2000_August_1/ai_63778269)

(34) Dawson R, Condos R, Lucke W, Ress S, Raju B, Bateman E, et al. Randomized clinical trial of aerosol or subcutaneous interferon gamma plus DOTS versus DOTS alone for cavitary tuberculosis: early results. *Am J Respir Crit Care Med* 2006;S2(A745).

(35) Johnson BJ, Bekker LG, Rickman R, Brown S, Lesser M, Ress S, et al. RhuIL-2 adjunctive therapy in multidrug resistant tuberculosis: a comparison of two treatment regimens and placebo. *Tuber Lung Dis* 1997;78(3-4):195-203.

(36) Johnson JL, Ssekasanvu E, Okwera A, Mayanja H, Hirsch CS, Nakibali JG, et al. Randomized trial of adjunctive interleukin-2 in adults with pulmonary tuberculosis. *Am J Respir Crit Care Med* 2003;168(2):185-91.

(37) Singh IG, Mukherjee R, Talwar GP, Kaufmann SH. In vitro characterization of T cells from *Mycobacterium w*-vaccinated mice. *Infect Immun* 1992;60:257-63.

(38) Singh IG, Mukherjee R, Talwar GP. Resistance to intravenous inoculation of *Mycobacterium tuberculosis* H37Rv in mice of different inbred strains following immunization with a leprosy vaccine based on *Mycobacterium w*. *Vaccine* 1991;9:10-4.

(39) Patel N, Deshpande MM, Shah M. Effect of an immunomodulator containing *Mycobacterium w* on sputum conversion in pulmonary tuberculosis. *J Indian Med Assoc* 2002;100:191-3.

(40) Wallis RS, Kyambadde P, Johanson JL, Horter L, Kittle R, Pohle M, et al. A study of the safety, immunology, virology, and microbiology of adjunctive etanercept in HIV-1-associated tuberculosis. AIDS 2004;18(2):257-64.

(41) Mayanja-Kizza H, Jones-Lopez E, Okwera A, Wallis RS, Ellner JJ, Mugerwa RD, et al. Immunoadjuvant therapy for HIV-associated tuberculosis with prednisolone: a phase II clinical trial in Uganda. *Journal of Infectious Diseases* 2005 15;191(6):856-65.

(42) Mitchison DA. Modern methods for assessing the drugs used in the chemotherapy of mycobacterial disease. *Journal of Applied Bacteriology* 1996;25:72-80S.

(43) Mitchison DA. Assessment of new sterilizing drugs for treating pulmonary tuberculosis by culture at 2 months. *American Review of Respiratory Disease* 1993;147(4):1062-3.

(44) Yew W, Chan F, Chau M, Tam C, Leung F, Wong P, et al. Outcomes of patients with multidrug-resistant pulmonary tuberculosis treated with ofloxacin/levofloxacin-containing regimens. *Chest* 2000;117(3):744-51.

(45) Montvale N. Priftin (rifapentine) package insert. Physician's desk reference. *Medical Economics* 1999;1334-8.

(46) Benator D, Bhattacharya M, Bozeman L, Burman W, Cantazaro A, Chaisson R, et al. Rifapentine and isoniazid once a week versus rifampicin and isoniazid twice a week for treatment of drug-susceptible pulmonary tuberculosis in HIV-negative patients: a randomised clinical trial. *Lancet* 2002;360(9332):528-34.

(47) Brindle R, Odhiambo J, Mitchison DA. Serial counts of Mycobacterium tuberculosis in sputum as surrogate markers of the sterilising activity of rifampicin and pyrazinamide in treating pulmonary tuberculosis. *BMC Pulmonary Medicine* 2001;1(1):2.

(48) Jindani A, Aber VR, Edwards EA, Mitchison DA. The early bactericidal activity of drugs in patients with pulmonary tuberculosis. *American Review of Respiratory Disease* 1980;121(6):939-49.

(49) Davies GR, Brindle R, Khoo SH, Aarons LJ. Use of nonlinear mixed-effects analysis for improved precision of early pharmacodynamic measures in tuberculosis treatment. *Antimicrob Agents Chemother* 2006;50(9):3154-6.

(50) Wallis RS, Patil S, Cheon SH, Edmonds K, Phillips M, Perkins MD, et al. Drug tolerance in *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother* 1999;43(11):2600-6.

(51) Heifets LB, Good RC. Current laboratory methods for the diagnosis of tuberculosis. In: Bloom BR, ed. Tuberculosis: pathogenesis, protection and control. 1st edition. Washington, DC, ASM Press, 1994.

(52) Wallis RS, Perkins MD, Phillips M, Joloba M, Namale A, Johnson JL, et al. Predicting the outcome of therapy for pulmonary tuberculosis. *Am J Respir Crit Care Med* 2000;161(4):1076-80.

(53) Epstein MD, Schluger NW, Davidow AL, Bonk S, Rom WN, Hanna B. Time to detection of Mycobacterium tuberculosis in sputum culture correlates with outcome in patients receiving treatment for pulmonary tuberculosis. *Chest* 1998;113(2):379-86.

(54) Hasegawa N, Miura T, Ishizaka A, Yamaguchi K, Ishii K. Detection of mycobacteria in patients with pulmonary tuberculosis undergoing chemotherapy using MGIT and egg-based solid medium culture systems. *The International Journal of Tuberculosis and Lung Disease* 2002;6(5):447-53.

(55) Prentice RL. Surrogate endpoints in clinical trials: definition and operational criteria. *Statistics in Medicine* 1989;8(4):431-40.

(56) Fleming TR, Prentice RL, Pepe MS, Glidden D. Surrogate and auxiliary endpoints in clinical trials, with potential applications in cancer and AIDS research. *Statistics in Medicine* 1994;13(9):955-68.

(57) Alonso A, Molenberghs G, Burzykowski T, Renard D, Geys H, Shkedy Z, et al. Prentice's approach and the meta-analytic paradigm: a reflection on the role of statistics in the evaluation of surrogate endpoints. *Biometrics* 2004;60(3):724-8.

(58) Freedman LS, Graubard BI, Schatzkin A. Statistical validation of intermediate endpoints for chronic diseases. *Statistics in Medicine* 1992;11(2):167-78.

(59) Day NE, Duffy SW. Trial design based on surrogate end points - application to comparison of different breast screening frequencies. *J R Statist Soc Series A* 1996;159:49-60.

(60) Fleming TR, DeMets DL. Surrogate end points in clinical trials: are we being misled? *Ann Intern Med* 1996;125(7):605-13.

(61) Begg CB, Leung DHY. On the use of surrogate end points in randomized trials. *J R Statist Soc Series A* 2000;163(1):15-28.

(62) Preliminary report: effect of encainide and flecainide on mortality in a randomized trial of arrhythmia suppression after myocardial infarction. The Cardiac Arrhythmia Suppression Trial (CAST) Investigators. *N Engl J Med* 1989;321(6):406-12.

Special Programme for Research & Training in Tropical Diseases (TDR) sponsored by
UNICEF/UNDP/World Bank/WHO

TDR/World Health Organization
20, Avenue Appia
1211 Geneva 27
Switzerland

Fax: (+41) 22 791-4854
tdr@who.int
www.who.int/tdr

ISBN 978 92 4 159583 4

The Special Programme for Research and Training in Tropical Diseases (TDR) is a global programme of scientific collaboration established in 1975. Its focus is research into neglected diseases of the poor, with the goal of improving existing approaches and developing new ways to prevent, diagnose, treat and control these diseases. TDR is sponsored by the following organizations:

unicef UNDP World Bank World Health Organization