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ABSTRACT

On July 9, 2014, Aeras and the Max Planck Institute for Infection Biology convened a workshop entitled "Whole Mycobacteria Cell Vaccines for Tuberculosis" at the Max Planck Institute for Infection Biology on the grounds of the Charité Hospital in Berlin, Germany, close to the laboratory where, in 1882, Robert Koch first identified *Mycobacterium tuberculosis* (Mtb) as the pathogen responsible for tuberculosis (TB). The purpose of the meeting was to discuss progress in the development of TB vaccines based on whole mycobacteria cells. Live whole cell TB vaccines discussed at this meeting were derived from Mtb itself, from Bacille Calmette–Guérin (BCG), the only licensed vaccine against TB, which was genetically modified to reduce pathogenicity and increase immunogenicity, or from commensal non-tuberculous mycobacteria. Inactivated whole cell TB and non-tuberculous mycobacterial vaccines, intended as immunotherapy or as safer immunization alternatives for HIV+ individuals, also were discussed. Workshop participants agreed that TB vaccine development is significantly hampered by imperfect animal models, unknown immune correlates of protection and the absence of a human challenge model. Although a more effective TB vaccine is needed to replace or enhance the limited effectiveness of BCG in all age groups, members of the workshop concurred that an effective vaccine would have the greatest impact on TB control when administered to adolescents and adults, and that use of whole mycobacteria cells as TB vaccine candidates merits greater support, particularly given the limited understanding of the specific Mtb antigens necessary to generate an immune response capable of preventing Mtb infection and/or disease.

1. Introduction

Dr. Stefan H.E. Kaufmann, Managing Director, Max Planck Institute for Infection Biology and Professor of Immunology and Microbiology, Charité Clinics, Berlin, Germany

The development of an effective vaccination to prevent the spread of tuberculosis (TB) represents an important global health priority. It is estimated that one third of the world's population is infected with *Mycobacterium tuberculosis* (Mtb). In 2013, approximately 9 million persons developed active TB, and approximately 1.5 million died of the disease [1]. In 2014, the WHO set a goal of reducing incidence of active TB from the current level of greater than 100 cases per 100,000 persons to 10 cases per 100,000 persons by 2035, and reducing mortality by 95% [1]. Models of disease reduction strategies suggest that current TB control strategies will not be sufficient to reach this goal unless a vaccine capable of preventing TB infection and/or disease becomes available [2–4].

Sixteen different TB vaccine candidates are currently in clinical trials, with more in the preclinical pipeline. Most of these vaccine candidates are subunit vaccines, where selected Mtb antigens are expressed in recombinant viral vectors or are administered as protein/adjuvant combinations [5]. Approximately 12 different antigens are expressed in the subunit vaccines currently in clinical trials. A major challenge to TB vaccine development, however, is the lack of an immune correlate of protection against Mtb infection or TB disease [6]. Accordingly, there is little certainty about the actual protective effect that may be provided by at least some of the antigens currently under investigation in subunit vaccine candidates [7].

Given these concerns, whole mycobacteria cell vaccines are receiving a fresh look as an attractive TB vaccine development strategy [8]. The most familiar whole cell vaccine for TB is Bacille Calmette–Guérin (BCG). Working in the Institute Pasteur in Lille, France, Albert Calmette and Camille Guérin passaged a *Mycobacterium bovis* isolate over a period of 16 years, from 1906 to 1921, eventually developing a strain sufficiently attenuated to administer safely to humans [9,10]. Since its first use in 1921, BCG has become the most widely used vaccine in history, with approximately 4

Abbreviations: AFB, acid-fast bacteria; BCG, Bacille Calmette–Guérin; CFU, colony forming units; DAT, diacyltrehalose; DDA, dimethyldioctadecylammonium; ELISA, enzyme-linked immunosorbent assay; ELISpot, enzyme-linked immunospot assay; HIV, human immunodeficiency virus; Hly, listeriolysin O; IFN-γ, Interferon gamma; INH, isoniazid; LAM, lipoarabinomannan; LTBI, latent TB infection; MDR, multidrug-resistant; MGIA, mycobacterial growth inhibition assay; MPL, monophosphoryl lipid-A; Mtb, *Mycobacterium tuberculosis*; NHP, non-human primate; NKT, natural killer T-cells; PAT, polyacyltrehalose; PDIM, phthiocerol dipimycocerosate; PPD, purified protein derivative; SATVI, South African Tuberculosis Vaccine Initiative; SL, Sulfolipid; TB, tuberculosis; TNF, tumor necrosis factor; VPM, Vakzine Projekt Management; WHO, World Health Organization; Zmp, zinc metalloprotease.

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billion doses administered worldwide [5]. The full effectiveness of BCG vaccination, however, has yet to be accurately determined. There is consensus that BCG administered to infants shortly after birth reduces the risk of severe childhood TB, particularly TB meningitis and disseminated TB. A meta-analysis of the global effect of BCG vaccination on childhood tuberculous meningitis and military TB estimated that in 2002, about 30,000 cases of tuberculous meningitis and 11,500 cases of military TB were prevented by the 100.5 million BCG vaccinations given to infants in that year [11]. Accordingly, the WHO recommends neonatal BCG immunization in countries endemic for TB [11,12]. BCG also may provide children a degree of protection against Mtb infection for a limited number of years after vaccination, although observational studies suggest that this effect is variable and likely is impacted by the latitude in which the child lives, with children in tropical latitudes exhibiting less protection from subsequent childhood infection, possibly due to a higher rate of exposure to other non-tuberculous mycobacteria [13,14].

Despite its widespread use, BCG vaccination has important limitations. Recent studies in BCG-immunized pediatric populations estimates total incident childhood cases of all forms of TB in 2011 to be almost 1 million, twice that estimated by WHO, and provides an initial assessment of multidrug-resistant (MDR) TB cases at over 30,000 [15]. In addition, BCG is not recommended for use in HIV-infected infants because of the risk of disseminated BCG disease [16]. Most importantly, the ongoing global epidemic of TB infection, disease and death among adolescents and adults is occurring in populations in which infant BCG vaccination is nearly universal. Accordingly, developing a more effective whole cell vaccine than BCG represents an important goal of global TB control efforts.

The July 9, 2014 whole mycobacteria cell TB vaccine workshop, convened in the shadow of the laboratory of Robert Koch, discoverer of the Mtb bacillus more than 130 years previously, specifically examined the potential role of whole mycobacterial cells as vaccines to prevent infection and/or disease due to Mtb. The workshop was divided into three sessions: (1) the research and development status of seven whole cell TB vaccines; (2) development pathways for whole mycobacteria cell vaccines, focusing on the rationale for and public health implications of developing replacement BCG vaccines for infants and for using whole cell vaccines to prevent Mtb infection and disease in adolescents and adults; and (3) resource needs for developing whole cell TB vaccines, with a focus on the identification of standardized assays for vaccine immunogenicity and efficacy to permit reliable vaccine candidate comparisons. A summary of the whole mycobacteria cell vaccines for TB discussed in this workshop can be found in Table 1.

2. Status of ongoing whole mycobacteria cell vaccine research and development

BCG + ESAT-6 recombinants. Dr. Roland Brosch, Institut Pasteur, Unit for Integrated Mycobacterial Pathogenomics, Paris, France

Dr. Roland Brosch described efforts by his laboratory to improve the BCG vaccine. Theorizing that BCG may be lacking some important genetic features to provide effective protection against Mtb, Dr. Brosch proposed that vaccine immunogenicity and efficacy may be improved by adding back a gene cluster to BCG that was lost during the BCG passaging and attenuation process. All BCG vaccines lack the RD1 locus, the region of difference 1 [17,18]. The RD1 locus encodes at least 11 genes, including the immunodominant T-cell antigens ESAT-6 (6-kD early secretory antigenic target) and CFP-10 (10-kD culture filtrate protein), both representing important mycobacterial antigens secreted by the ESX-1 type VII secretion system, representing potential Mtb virulence factors. Restoration of the entire RD-1 locus to BCG is required for ESAT-6 secretion and partially restores virulence [18]. When the recombinant BCG, BCG::ESX-1, was compared to the parental BCG for immunogenicity and protection against an Mtb challenge [19], it was shown to be more virulent in immune deficient mice, more persistent in immune competent mice, but also more effective in protecting against disseminated TB in both mice and guinea pigs [18,19].

As a possible explanation for the increased virulence in severe combined immune deficient (SCID) mice and the improved protective vaccine efficacy of the recombinant strain, it was found that ESAT-6 may disrupt lipid bilayers [20,21], thereby providing a mechanism for ESX-1 proficient tubercle bacilli to egress from the macrophage phagosome to the cytoplasm [22,23]. The effect of ESAT-6 on the fate of macrophage-phagocytized BCG also was studied with the fluorescent substrate, CCF-4. BCG is unable to progress from the phagosome to the cytoplasm, while BCG::ESX-1 moves to the cytoplasm with Mtb kinetics. Phagosomal rupture is followed by necrotic cell death of infected macrophages, resulting in BCG::ESX-1spread to new host cells. Access to the cytosol by the recombinant BCG::ESX-1 allows additional immunological responses and higher amounts of antigen, in part also explaining the stronger $CD8^+$ T-cell responses observed for Mtb relative to BCG [24].

A series of recombinant, ESX-1 proficient BCG constructs were developed that retain ESX-1 function but reduce the virulence. Mutation of ESAT-6 leads to less virulent recombinant BCG vaccines that express and secrete modified ESAT-6 antigens which proved to provide better protection in mouse models and to be somewhat more protective in a guinea pig model than the parental BCG Pasteur strain (Bottai and Brosch, unpublished results). The use of different BCG strains as a template for ESX-1 complementation, such as BCG Moreau, also is being explored. Finally, attempts also are being made to use the recombinant BCG::ESX-1 Mar, created using the ESX-1 system from *Mycobacterium marinum*, a biosafety class 2 organism, to complement BCG strains. Preliminary results for this approach are promising (Groschel and Brosch, unpublished results). Dr. Brosch concluded his presentation by emphasizing that the ESX-1 locus encodes genes critical for mycobacterial host pathogen interaction, and that inclusion of this locus in recombinant BCG vaccines is expected to enhance the immune response to the vaccine on multiple levels which seem all to be impacted by the ability of the vaccine to gain access to the host cytosol [25].

VPM1002. Dr. Leander Grode, Vakzine Projekt Management GmbH, Hannover, Germany. Dr. Umesh Shaligram, Serum Institute of India Ltd., Pune, India

Dr. Leander Grode and Dr. Umesh Shaligram jointly discussed progress in scaling up production of the vaccine candidate VPM1002, and shared the results of Phase 1 and Phase 2 clinical trials. VPM1002 employs the pore-forming protein listeriolysin O (Hly) from the facultative anaerobic bacterium *Listeria monocytogenes*, coupled with a urease C gene deletion in a BCG Prague genetic background, to allow antigen to escape from the phagosome into the cytoplasm of the infected cell [26]. The BCG bacteria, however, remain in the phagosome [26]. Acidic pH is optimal for Hly activity; deletion of the urease C gene prevents BCG neutralization of the naturally acidic phagosome environment [27]. A safety feature of this vaccine is the four amino acid PEST sequence (P, proline; E, glutamic acid; S, serine; T, threonine) which targets the Hly protein for rapid degradation in the cytosol [28,29]. Following entry into the cytosol, the Hly protein is rapidly inactivated, thereby reducing the possibility of adverse events due to the pore-forming bioactivity of this protein. Antigen access to the cytosol is presumed to allow for a broader activation of immune mechanisms and improved vaccine efficacy [30–33].

VPM1002 initially was created in the laboratory of Dr. Stefan H.E. Kaufmann [34,35] and was further developed by VPM. In 2013,

Table 1
Whole mycobacteria cell vaccines for tuberculosis under development.

Vaccine	Description	Indication	Clinical trials	Comments
Recombinant BCG	BCG, essential virulence genes (e.g., ESAT-6) restored	BCG replacement	None	Early preclinical studies to date
VPM1002	BCG, *listeriolysin O* gene added, urease C gene deleted	BCG replacement; disease prevention in BCG vaccinated adults; bladder cancer	Phase 1 and 1b completed. Phase 2 in neonates ongoing	Phase 3 in HIV+/− neonates in planning
MTBVAC	*M. tuberculosis*, live, attenuated via deletions in *phoP* and *fadD26*	BCG replacement	Phase 1 in progress	Phase 2 in neonates planned for 2015
DAR-901	*Mycobacteria obuense*, heat inactivated, sonicated	Disease prevention in BCG vaccinated adults, especially HIV+	Phase 1, 2, 3 studies with agar-grown SLR-172 precursor to DAR-901 completed. Phase 1 with reformulated, broth-grown DAR-901 in progress.	Phase 3 planned in HIV+/− adults; Inactivated vaccine, safe for HIV+
RUTI	*M. tuberculosis*, fragmented, Triton cleaned, liposomal grown under anoxic stress	Immunotherapy to support and reduce TB and LTBI chemotherapy	Phase 1 and 2 completed	Two Phase 3 trials planned; not a live vaccine, safe for HIV+
BCG *Δzmp1*	BCG, zinc metallo-protease 1 deletion (*zmp1*)	BCG replacement	None	Late preclinical development
Adjuvanted, non-replicating BCG	BCG, *ΔpanCD*, auxotrophic for pantothenate	Boost after BCG prime in adolescents, adults and HIV+	None	Potentially more reactogenic than BCG

the rights to the vaccine were acquired by SII, which has developed a new large-scale production process for the vaccine. VPM1002 is being developed primarily as a BCG replacement vaccine for prevention of serious TB disease in infants. It also is being evaluated as a replacement for BCG immunotherapy of bladder cancer.

Preclinical studies in mice showed that, in contrast to BCG, VPM1002 reproducibly protected against an aerosol challenge model to a clinical isolate of Mtb of the Beijing/W lineage [26]. Safety assessment of bacilli persistence in wild type mice found that VPM1002 fell below the level of detection 40 days after immunization while BCG was detectable 90 days after immunization, The first Phase 1 clinical trial of VPM1002 was completed in Germany in 2012 [36]. The study consisted of adult male Caucasian volunteers with and without prior exposure to BCG. It was shown to be safe and similar in immunogenicity to BCG [36]. A subsequent Phase 1B trial in South Africa tested VPM1002 in BCG immunized adults. VPM1002 again was found to be safe and immunogenic as a boost to pre-existing immunity from a BCG prime [27,36]. A Phase 2, head-to-head comparison of BCG to VPM1002 in HIV-unexposed neonates recently was completed in South Africa. VPM1002 was at least as well tolerated as BCG in this trial; immunogenicity results are pending. A second Phase 2 clinical trial is planned to begin in January 2015 in South Africa in HIV-exposed and -unexposed populations of BCG-naïve neonates.

MTBVAC. Dr. Carlos Martín, Department of Microbiology, Faculty of Medicine, University of Zaragoza, Zaragoza, Spain

Dr. Carlos Martin reported on the development of MTBVAC, the only live-attenuated Mtb-based vaccine currently in clinical trials [37]. The rationale behind the development of an attenuated Mtb vaccine is to immunize with a candidate that manifests as close an antigenic picture to Mtb as possible while minimizing the virulence.

The Geneva Consensus on new live mycobacterial vaccines requires two non-reverting independent mutations to live, Mtb-based vaccines to insure the stability of the attenuation, without the inclusion of antibiotic resistance markers [38,39]. MTBVAC meets these requirements as it contains deletions in the genes encoding fadD26 and phoP and does not contain an antibiotic resistant marker. The fadD26 gene product is involved in the synthesis of the lipid phthiocerol dipimycocerosate (PDIM), a major mycobacterial virulence factor. The phoP gene encodes a transcriptional regulator that controls the expression of two percent of the coding capacity of Mtb genome, including gene families involved in respiration, the hypoxic response, lipid metabolism, stress proteins and the RD1 region, which includes ESAT-6 [40]. The deletion in phoP reduces the virulence mainly from abrogation of the ESX-1 secretion system. As a result of this deletion, MTBVAC maintains the ability to produce ESAT-6 but cannot secrete this, therefore losing the ability to spread from cell to cell [41]. In addition, the phoP deletion results in the loss of MTBVAC complex lipids, such as diacyltrehaloses (DAT), polyacyltrehaloses (PAT) and sulfolipids (SL), that could interfere with the immune system [40,42]. The resulting vaccine is an attenuated Mtb that is transformed from an immune-evasive to an immune-recognizable bacterium. Preclinical studies in mice demonstrated better protection than BCG [37,43]. Additionally, MTBVAC performs as well or better than BCG in non-human primates (NHPs) [44]. MTBVAC efficacy may be ascribed to improved antigenicity properties following silencing of the noncoding RNA Mcr7 and the ensuing increase in secretion of Twin Arginine Translocation (Tat) substrates, such as the Ag85 proteins, considered as major antigens of Mtb [42]. Phase 1 clinical trials designed to compare MTBVAC to BCG were initiated in January 2013 in Switzerland, in healthy adults negative for PPD, BCG and HIV. The vaccine was safe at all three doses tested; immunogenicity results are not yet available. In addition to standard immunogenicity assays, a transcriptomics study will be performed to better understand the molecular basis of the immune response to the vaccine. A Phase 1b clinical trial in neonates is planned for 2015.

DAR-901. Dr. C. Fordham von Reyn, Infectious Disease and International Health, Geisel School of Medicine, Hanover, New Hampshire

Dr. Ford von Reyn reported on the development of DAR-901, a vaccine designed to be safe and effective in both HIV-infected and -uninfected individuals. DAR-901 is a poly-antigenic, heat-inactivated, whole cell vaccine derived from an environmental non-tuberculous mycobacterium, *Mycobacteria obuense*, and manufactured using broth culture methods. Environmental non-tuberculous mycobacteria share multiple antigens with Mtb and may therefore provide cross protection, providing the rationale for the development of this vaccine candidate.

SRL-172 is an agar-grown precursor to the DAR-901 vaccine. Phase 1 and Phase 2 clinical trials in HIV-infected and HIV-uninfected volunteers with SRL-172 have been conducted in the USA, Zambia [45] and Finland [46]. The DarDar trial, a Phase 3

efficacy trial of SRL-172, was a randomized, placebo-controlled, double-blind trial in more than 2000 HIV-infected volunteers in Tanzania from 2001 to 2008. HIV-infected adults, with no active TB, and with prior childhood BCG immunization and $CD4^+$ T-cell counts greater than 200, received either five intradermal doses of the vaccine or an identical placebo over a 12-month period. Primary outcome measures were the prevention of disseminated and pulmonary TB. Subjects were followed every three months for a median of 3.3 years for evidence of TB disease. The trial was stopped in Year 7 by the Data and Safety Monitoring Board because the vaccine was judged to have shown efficacy in TB prevention. Definite TB, defined by ≥1 positive blood culture for Mtb, ≥1 positive sputum culture(s) with ≥10 colony-forming units (CFU), ≥2 positive sputum cultures with 1–9 CFU, ≥sputum smears with ≥2 acid fast bacilli (AFB)/100 oil immersion fields, or ≥1 positive culture, or positive AFB smear and caseous necrosis from a sterile site (other than blood), was statistically significantly reduced by 39% [47]. Disseminated TB was reduced by 50% but was underpowered for statistical significance. Immunogenicity of the vaccine was demonstrated by IFN-γ and lymphocyte proliferative responses in PMBC to vaccine sonicate as well as the increased presence of antibody directed against lipoarabinomannan (LAM), a glycoprotein widely expressed in mycobacteria [48]. No severe adverse reactions were seen, but reactions at the vaccine site were common [47].

In 2011, in collaboration with Aeras, a high yield and scalable GMP broth manufacturing process was developed from the Master Cell Bank for SRL-172 to produce DAR-901. Following the completion of animal toxicology and immunogenicity studies, a randomized, placebo controlled, dose escalation Phase 1 trial of DAR-901 was initiated in April 2014 in the USA with HIV-infected and HIV-uninfected volunteers, all of whom had previously received BCG. Immune assays will be conducted on all subjects to compare immunogenicity of the broth-grown DAR-901 to data from subjects who received agar-grown SRL-172 in earlier trials.

DAR-901 is intended to be a booster vaccine for the prevention of TB in both HIV-infected and HIV-uninfected adolescents and adults primed by childhood BCG immunization. Two Phase 3 trials in adolescents and adults in TB endemic countries are in planning for implementation upon successful completion of the ongoing Phase 1 trial: a multi-site trial in Tanzania with HIV-infected individuals and a multi-site trial in India and China with HIV-uninfected individuals.

RUTI. Dr. Pere-Joan Cardona, Unitat de Tuberculosi Experimental, Universitat Autónoma de Barcelona, Badalona, Catalonia, Spain

Dr. Pere-Joan Cardona described progress in the development of RUTI, an immunotherapeutic TB vaccine being developed by Archivel Farma, Catalonia, Spain, designed to shorten treatment of latent TB infection (LTBI), support chemotherapy of active TB disease and reduce recurrence rates following completion of treatment. The ideal RUTI strategy would reduce 9 months of isoniazid (INH) treatment of LTBI to only 1 month of INH combined with two doses of RUTI.

RUTI is fragmented, Triton cleaned, liposomal Mtb which has been cultured under stress conditions to induce latency antigens. The vaccine is detoxified with Triton to decrease the risk of the Koch reaction, an exacerbated immune reaction that may occur when persons already infected with Mtb are exposed to Mtb antigens via vaccination, and contains very low levels of the endotoxin-like molecule, LAM. The liposomal preparation is stable at room temperature and displays both adjuvant and antigen properties [49]. In a mini-pig model of latent TB, combined INH and RUTI treatment reduced the ratio of new to old lesions when compared to untreated controls and INH alone [50].

A Phase 1, randomized, placebo-controlled clinical trial of RUTI was conducted in healthy white males in Spain with no prior BCG vaccination or history of TB infection to evaluate the tolerability and immunogenicity of four RUTI doses between 5 μg and 200 μg. Results obtained following the completion of the trial in October 2008 demonstrated that all four doses were tolerated, but higher doses (100 μg and 200 μg) were accompanied by moderate pain (20 on a scale of 100) at the injection site, which was more intense after the second inoculation. Specific immune responses against PPD and four specific Mtb antigens were detected 35 days after vaccination [51].

A Phase 2 study of RUTI safety, tolerability and immunogenicity was completed in July 2011. It was a randomized, placebo-controlled trial in HIV-infected and HIV-uninfected patients undergoing INH treatment for LTBI. Four weeks of INH treatment was followed by two RUTI inoculations administered 28 days apart; 5 μg, 25 μg and 50 μg doses of RUTI were compared to placebo. More than ninety percent of patients experienced injection site reaction, including erythema, induration and nodulation lasting 3–4 weeks, but self-resolved in almost all cases. The moderate local pain was well tolerated and had the same intensity as was observed in the Phase 1 study. There was no evidence of a Koch reaction. After this trial, the RUTI production process has changed to include a filtration step in an effort to reduce adverse events at the inoculation site. Immune response to RUTI was variable; frequently the TB ELISpot IFNγ response for both individual TB antigens and for PPD in the experimental samples was polyantigenic and higher than placebo and was weaker in HIV-infected than in HIV-uninfected subjects [52] Using a whole blood, seven-day ELISA assay for IFNγ to gauge response to PPD, the 25 μg RUTI dose consistently showed an increase in IFNγ production versus placebo with an approximately seventy-five percent response rate in both HIV-infected and HIV-uninfected subjects [52]. Based on the Phase 2 results, the developers designed Phase 3 clinical trials to include a single, 25 μg injection of RUTI.

Two Phase 3 clinical trials are in planning to demonstrate RUTI efficacy in reducing relapse after chemotherapy for LTBI or active TB disease. One employs a single, 25-μg injection of RUTI or a placebo after 6 months of INH treatment for LTBI in HIV-infected individuals where RUTI is designed to add at least 60% protection against relapse. The second Phase 3 trial tests the relapse protection provided by a single, 25-μg injection of RUTI versus placebo three months into six months of standard chemotherapy for drug-susceptible active TB.

BCG Δ*zmp1*. Dr. Peter Sander, Institute of Medical Microbiology, University of Zurich, Zurich, Switzerland

Despite extensive attenuation, BCG continues to retain many of the immune evasive properties of Mtb, which may limit its protective efficacy. Zinc metalloprotease 1 (*zmp1*) has been identified as one mediator of immune evasion common to both Mtb and BCG [53,54]. The enzyme enhances bacterial survival in the host macrophage by preventing phagosome–lysosome fusion and inflammasome activation, allowing the bacteria to evade major histocompatibility class I- or II-restricted T-cell responses [55,56]. Dr. Peter Sander presented an update on the development of a BCG strain in which the *zmp1* gene has been deleted, BCG *Δzmp1*. The common general rationale shared by BCG *Δzmp1*, the recombinant BCG constructs described by Dr. Brosch, and VPM1002 is to overcome mycobacterial immune evasion in the macrophage phagosome, albeit by different mechanisms

Preclinical studies in mice have shown that BCG *Δzmp1* is more immunogenic than BCG. Despite the improved immunogenicity, however, there was no statistical difference between the *zmp1* deletion mutant and wild type BCG persistence in immune competent mice [56]. Studies of the safety of *zmp1*-deleted BCG strains in SCID mice suggest that the *zmp1* deletion is typically safer than the isogenic parental strain, whether it is Danish or Pasteur.

BCG Δzmp1 has been found to be protective and safe in animal challenge experiments conducted thus far. Low-dose challenge studies in guinea pigs, utilizing 10–50 CFU of the virulent Mtb strain H37Rv administered via aerosol, demonstrated that the protective efficacy of BCG *Δzmp1* in either the Danish or Pasteur BCG strain is superior to standard BCG in reducing the number of tubercle bacilli in the lung [57]. In cattle, BCG *Δzmp1* induces stronger memory responses than standard BCG, which has been described as the most predictive indicator of protective efficacy [58]. Challenge studies in cattle, comparing BCG *Δzmp1* to BCG, are in progress.

BCG ΔPanCD (BCG Aeras Pasteur). Dr. Barry Walker, Vice President, Preclinical Development, Aeras, Rockville, Maryland, USA

Dr. Barry Walker discussed a vaccine strategy that combines a non-replicating BCG with adjuvant. This strategy is intended to develop a safer BCG vaccine for use as a booster in previously BCG-vaccinated adolescents, adults and immune-compromised individuals. BCG ΔPanCD, designated BCG Aeras Pasteur, is auxotrophic for pantothenate (Vitamin B5) and requires supplementation with pantothenate for growth. Coupled with one of a number of state-of-the-art adjuvants, the resulting vaccine could be both safer and more effective than BCG. Studies in a mouse challenge model have shown BCG Aeras Pasteur to be as protective as replicating BCG, while BCG adjuvanted with dimethyldioctadecylammonium liposomes with monophosphoryl lipid-A (DDA/MPL) may be more protective than BCG alone (Walker, unpublished results). Additional studies are underway to confirm this observation.

3. Development pathways for whole mycobacteria cell TB vaccines: BCG replacement in infants and potential for use in adolescents and adults

Dr. Michele Tameris, University of Cape Town, South African Tuberculosis Vaccine Initiative (SATVI), Cape Town, South Africa

Dr. Richard G. White, TB Modeling Group, Department of Infectious Disease Epidemiology, London School of Hygiene and Tropical Medicine, London, UK

An important issue facing developers of whole mycobacteria cell vaccines for TB is whether to develop them primarily as possible replacements for BCG administered to infants and young children to prevent TB meningitis or disseminated TB, or whether to target their development as vaccines that could contribute to efforts to develop vaccine regimens to prevent Mtb infection or pulmonary TB disease in adolescents and adults. Dr. Michele Tameris began her rationale in support of the BCG replacement strategy by noting that, overall, BCG is known to display widely variable protection against pulmonary TB, varying between 0 and 80%. In a 1995 meta-analysis, BCG was found to have an overall protective effect of 74% in newborns and infants [59]. In a recent, comprehensive meta-analysis undertaken in an attempt to explain the wide variation in BCG efficacy, an average of 59% protection for pulmonary TB and 85% protection against meningeal and miliary TB was seen in infants in studies initiated between 1933 and 1988 [14]. Additionally, a study of the cost effectiveness of BCG in preventing childhood tuberculous meningitis and miliary TB demonstrated that the 100.5 million BCG vaccinations administered in 2002 were estimated to have prevented almost 30,000 cases of tuberculous meningitis and about 11,500 cases of miliary TB making it a highly cost-effective intervention against severe childhood TB [11].

Despite evidence of BCG efficacy in young children, however, BCG vaccinated children continue to experience high rates of TB in endemic areas. Additionally, BCG is not recommended for use in HIV-infected infants due to the risk of disseminated BCG disease [16]. More than 90% of infants in Cape Town are immunized with BCG, yet the incidence of the spectrum of TB disease in children under 2 years exceeded 1200 per 100,000 in the period between July 2002 and June 2003. Prior to the development of drug therapy for TB, the highest risk of TB mortality following primary infection in children under 15 years of age was 12.1%, occurring in children between the ages of 1 and 4 [60]. In addition, miliary TB and tuberculous meningitis is concentrated in children under 3 years of age, as found in a prospective, descriptive study conducted in Cape Town over a 20-month period between 2003 and 2004 [61]. Dr. Tameris cited these data when calling for a better vaccine for infants than BCG, one safe enough to use in children with HIV infection and other immunosuppressive conditions and which provides increased protection for all forms of TB in children.

Dr. Tameris reviewed suggested approaches to reduce the risk to infants in areas of high TB endemnicity enrolled in trials of new, modified BCG vaccines. To minimize the risk of Mtb infection or TB disease, neonates will be recruited from mothers who are free of both active and latent TB (i.e., QuantiFERON® negative). The infants and their household contacts will be subjected to active surveillance for symptoms of TB disease following immunization. Stringent halting rules will be adhered to, and a rescue dose of BCG made available if necessary at 6 months for Phase 1B trials and at 12 months for Phase 2A and 2B trials. Safety data still needs to be generated for delayed or repeat BCG immunization in infants.

In the discussion that followed Dr. Tameris's presentation, workshop participants expressed concern that demonstration of superiority of a new, modified BCG vaccine to standard BCG would be extraordinarily difficult and would require an extremely large study population. While a new BCG vaccine that prevents pulmonary TB in adolescents and adults would reduce Mtb transmission and therefore would eventually protect children, Dr. Tameris emphasized that children need primary protection dedicated to their age group now. BCG immunization after birth is systematic, routine and provides excellent coverage; a booster vaccine to enhance BCG-primed protection would keep the current neonatal BCG immunization system intact.

Dr. Richard G. White presented a case for targeting the development of whole mycobacterial cell vaccines for TB at adolescents and adults to achieve the greatest epidemiological impact before 2050. He based his conclusion on the results of a modeling study on the impact and cost-effectiveness of future TB vaccines in low- and middle-income countries [3]. More than 80% of the 8.6 million new cases of TB diagnosed in 2012 occurred in low- and middle-income countries. Without an effective vaccine, modeling studies have shown that global epidemiological goals for TB control by 2050 are unlikely to be achieved [2,4]. Dr. White and colleagues fitted variable levels of vaccine coverage, efficacy and duration of protection for different target populations into a standard TB transmission model. Age, HIV status and more effective future TB control was modeled. Impact and cost effectiveness was determined by comparing the impact and cost of TB control with and without a new vaccine. New vaccines were assumed to prevent active disease but not infection, to be effective in uninfected and infected individuals, and to require two doses. Immunization coverage was assumed to be 82% (47–99%) for infants, 70% (42–99%) for 10-year-old school children and 74% (68–91%) for mass vaccination campaigns. Efficacy was varied between 40%, 60% and 80%, and included the assumption that efficacy in HIV-infected individuals was 40% lower than in HIV-uninfected people. Duration was varied between 5 years, 10 years and lifelong protection. Finally, the price per dose was determined through historical analysis of GAVI prices of new vaccines and consultation with Aeras and TBVI. This model's results are in addition to the impact of BCG immunization at current coverage levels.

In lower income countries, with a vaccine that provided 40% efficacy over 10 years, over the 26 years between 2024 and 2050 the

model suggests that only about 0.6 million cases, or 2% of the total burden, would be averted if the vaccine was targeted at infants. In contrast, a vaccine with the same efficacy and duration profile would avert 13 million cases, or 40% of the total burden, if targeted at adolescents and adults.

A cost-effective vaccine price analysis demonstrated that a new vaccine targeted at adolescents and adults would still be cost effective if the duration of effectiveness was as short as five years and the efficacy was as low as 20%. New vaccines with long durations targeted at infants could only become cost-effective if the time endpoint extended beyond 2050. These results imply that new vaccines targeted at adolescents and adults could have a large public health impact and be cost effective, even if only short duration and low efficacy vaccines are available.

It will be critical in designing BCG replacement studies to take into account possible non-TB-related effects of BCG, including potential effects on all-cause mortality in the first few months after vaccination, potential effects on Th2 driven-conditions such as asthma, and studies to make sure the protective effect of BCG against leprosy is not lost [62,63].

4. Resource needs

Dr. Thomas Scriba, University of Cape Town, South African Tuberculosis Vaccine Initiative (SATVI), Cape Town, South Africa

Dr. David Hokey, Senior Director of Immunology and Animal Studies, Aeras, Rockville, Maryland, USA

As TB vaccines candidates enter clinical trials, common and standardized assays for immunogenicity and efficacy are needed to permit a rational selection of the most promising candidates to advance into later-stage efficacy trials [64]. Assay development has been limited by both the lack of a human challenge model for TB and an incomplete understanding of the immune correlates of protection. Dr. Thomas Scriba began his presentation on the selection of immune assays for whole mycobacteria cell vaccine candidates by noting that recent advances in our understanding of protective immunity to TB reveal a complex interplay between many cell types of both the innate and adaptive immune systems [65]. Whole mycobacteria cell vaccines provide a broad spectrum of antigens, including lipid and phospho-antigens in addition to protein antigens, and generally induce better immunological memory than subunit vaccines, potentially increasing the complexity of assays required to fully evaluate resultant immunogenicity. The non-specific protection against infection and mortality that whole mycobacteria cell vaccines may provide may further increase the challenges in developing appropriate assays to assess the full scope of the effects of these vaccines [66,67].

Assays should identify the immune cells that respond to the specific antigenic challenges provided by these vaccines, and demonstrate the memory state of the cells and the functional consequence of the response. The BCG-associated $CD4^+$ T-cell response peaks 6–10 weeks after vaccination in BCG naïve infants [68], but older populations display a much more variable response depending on their exposure history to BCG, TB and environmental mycobacteria. Potential attributes of the response to follow include the de novo proliferative responses of BCG-specific $CD4^+$ T-cells in infants, de novo $CD4^+$ T-cell expression of cytotoxic responses in infants, and increases in populations of conventional $CD8^+$ T-cells, as well as the more unconventional γδ T-cells, CD3-$CD56^{hi}$ NK cells, CD3-$CD56^{int}$ NK cells, and $CD3^+CD56^+$ NKT-cells. Mycobacterial growth inhibition assays (MGIAs) may measure a unique functional immunity that most accurately assesses vaccine-induced protection [69].

Dr. Scriba concluded his presentation by saying that assays should capture the breadth of the immune cells and effector functions induced by the vaccine. He cautioned that commonly used assays, such as soluble cytokine release assays and the ELISpot assay, may be too simple and univariate to provide sufficient information. Assays potentially capable of capturing relevant complexity include intracellular cytokine/cytotoxic staining and proliferation assays visualized through flow cytometry, and tetramer assays to capture the antigenic complexity of $CD4^+$ T-cells, $CD8^+$ T-cells, as well as CD1- and MR1-restricted T-cells. MGIAs may be the best overall measure of effector function. Variations in results due to the use of fresh or cryopreserved cells, whole blood, or peripheral blood mononuclear cells continue to be determined. Antibody responses may also be a valuable indicator of protective immunity but are as yet undefined. More predictive assays for protective immunity against TB will require future studies that address the still incompletely understood immunological mechanisms involved in response to Mtb infection and TB vaccine candidate administration.

Dr. David Hokey discussed the reagents needed to differentiate and compare whole mycobacteria cell TB vaccine candidates. Many challenges confront TB vaccine development, including the dearth of understanding as to the actual components of a protective human immune response to Mtb infection and TB disease. Animal challenge studies can be long and expensive and the correlation between pre-clinical studies in animal models and human protection is uncertain. Assays may need to be designed to assess the specific advantages of these candidates over BCG, such as the ability to induce greater $CD8^+$ T-cell responses, a greater shift toward Th1 cytokine production, and recognition of new antigenic epitopes induced by non-tuberculous mycobacteria. Despite these challenges, some progress has been made in identifying the required immune responses for TB control. These include identification of the critical role in TB control played by $CD4^+$ and $CD8^+$ T-cells, IFN-γ pathways and TNF. Work continues on the refinement of animal models, the expansion of immune assays to report on the immune response with greater complexity, and the growth of the clinical pipeline of new TB vaccines. Unfortunately, no clear correlate has been identified for the positive, non-specific effects of BCG, with the exception of one epigenetic marker [70].

The global clinical pipeline of sixteen TB vaccine candidates includes five whole mycobacteria cell vaccines, four which were presented at this meeting (three of the seven whole cell vaccines discussed in Session 1 are in the preclinical stage of development). The potential advantages of whole mycobacteria cell vaccines have been emphasized throughout this meeting, and include their broader antigen coverage, the natural adjuvanting properties of these cells, and their ease of manufacture. Disadvantages include the concern that since development of active TB may not provide immune protection against future infections, whole mycobacteria cell vaccine responses may not be able to provide protection against Mtb infection or TB disease.

A major challenge to conducting trials of whole mycobacteria cell vaccines is the need to accurately identify vaccine strains from naturally occurring Mtb as well as from environmental non-tuberculous mycobacteria, ubiquitous, soil-based organisms particularly common in tropical and subtropical regions. It will be critically important to develop accurate, standardized assays capable of easily and quickly distinguishing between mycobacterial species. Additionally, for adolescents and adults living in tropical and subtropical regions in particular, continual exposure to non-tuberculous environmental mycobacteria makes distinguishing vaccine responses from responses to other mycobacteria difficult [13].

Most immune assays for measuring responses to vaccines, whether simple assays measuring a single variable, or with more complex outcomes, require antigen for in vitro stimulation. Standardized reagents from a common source would allow

comparability between clinical trials. Possible antigen sources include the vaccine itself; PPD, protein antigens or peptides; or Mtb lysate.

Research resource needs were identified in several areas: expanding current assays beyond the Th1 response to improve measurements of immunity; using systems biology to identify correlates of protection; and developing further disease models, including an animal transmission model and a human challenge model.

5. Summary panel discussion

Panel members:

Dr. Barry Bloom Harvard University Distinguished Service Professor and Joan L. and Julius H. Jacobson Professor of Public Health, Department of Immunology and Infectious Diseases, Department of Global Health and Population, Harvard School of Public Health, Boston, MA, USA.
Dr. Hazel Dockrell, Professor of Immunology, London School of Hygiene and Tropical Medicine, London, UK.
Dr. Bernard Fritzell, Tuberculosis Vaccine Initiative (TBVI), Lelystad, the Netherlands.
Dr. Willem Hanekom, Tuberculosis/Global Health, Bill and Melinda Gates Foundation, Seattle, Washington, USA.
Dr. Stefan H.E. Kaufmann, Managing Director, Max Planck Institute for Infection Biology and Professor of Immunology and Microbiology, Charité Clinics, Berlin, Germany.
Dr. Michael Levin, Director, Wellcome Center for Clinical Tropical Medicine, Imperial College, London, UK.
Dr. January Weiner, Department of Immunology, Max Planck Institute for Infection Biology, Berlin, Germany.

In the concluding panel discussion, Dr. Hazel Dockrell emphasized the importance of developing at least one validated assay, using a standardized protocol, equipment and reagents, to be used in all clinical TB vaccine trials, especially in multi-center trials of a single vaccine. Clinical studies of whole mycobacteria cell vaccines should undertake a broad and complex analysis of the immune response, utilizing a variety of assays performed in as standardized a way as possible. Systems biology investigations represent powerful new strategies to identify possible immune correlates of protection [6]. Systems biology utilizes gene expression methods to identify a signature of immune response-associated gene activity accompanying certain clinically identifiable events, such as a transition from latent Mtb infection to active TB disease. Dr. Dockrell noted that it may be easier to identify biosignatures indicative of early clinical disease than those associated with protection from disease. In addition, systems biology whole blood studies primarily assess immune responses occurring within the vascular space rather than in the pulmonary parenchyma, the actual site of Mtb infection and most TB disease.

In light of the number of vaccine candidates to be developed and the enormous expense of clinical efficacy trials, Dr. Barry Bloom emphasized the urgent need for innovative strategies to measure effectiveness prior to committing to large scale vaccine trials. Systems biology approaches to finding gene expression signatures offer hope for predicting risk for progression to disease and possibly defining biomarkers of protection. Noting several fundamental differences in responses between humans and experimental animals to Mtb and citing the success of live challenge strategies that have been valuable for evaluating vaccines for malaria and enteric diseases, Dr. Bloom urged serious attention be given to the development of auxotrophic or conditional promotor-regulated Mtb strains that could be used as safe, live TB challenges in humans that would establish the ability of vaccine candidates to induce Mtb killing.

Dr. Stefan H.E. Kaufmann concluded the presentations by noting that the development of many whole mycobacteria cell TB vaccines are advancing, yet a great amount of work remains to be done in assessing the ultimate value of this vaccination strategy. While BCG replacement in infants has value it will not be easy to accomplish given the challenges inherent in comparing a new vaccine with an existing, safe product known to provide the only established efficacy against TB disease. Opportunities should be sought to diversify the use of whole mycobacteria cell TB vaccines in preventing infection and disease in adolescents and adults. Moreover, different vaccination schedules should be explored such as whole-cell vaccination alone or as a prime followed by a boost with a different platform, for example, vector-delivered vaccines or protein–adjuvant combinations. Similarly, different delivery modalities should be explored such as aerosol delivery in addition to intramuscular or intradermal application. Utilization of adaptive trial designs, allowing efficient modification of the clinical trials according to new data arising, should be considered. Additionally, samples from ongoing clinical studies should be collected and stored to gain deeper insights into the host response evoked by the vaccine undergoing clinical testing and to provide information to guide future vaccine design. Until and unless specific antigens are identified that prompt a protective immune response to Mtb, whole mycobacteria cell vaccines will continue to provide a critically important strategy in efforts to develop a vaccine against the global public health scourge of TB. To Dr. Kaufmann, success of a full development of an efficacious and safe TB vaccine will only be possible if all stakeholders share their knowledge and wisdom and work together as closely as possible.

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