Recent developments in tuberculosis vaccines

Dessislava Marinova, Jesus Gonzalo-Asensio, Nacho Aguilo and Carlos Martin

1 Grupo de Genética de Micobacterias, Dpto. Microbiología, Medicina Preventiva y Salud Pública, Universidad de Zaragoza, C/ Domingo Miral s/n, 50009 Zaragoza, Spain
2 CIBER Enfermedades Respiratorias, Instituto de Salud Carlos III, Madrid, Spain
3 Servicio de Microbiología, Hospital Universitario Miguel Servet, ISS Aragón, 50009 Zaragoza, Spain
*
Author for correspondence:
Tel.: +34 97 676 1759
Fax: +34 97 676 1664
carlos@unizar.es

Substantial efforts have been made over the past decade to develop vaccines against tuberculosis. We review recent developments in tuberculosis vaccines in the global portfolio, including those designed for use in a prophylactic setting, either alone or as boosts to Bacille Calmette–Guérin, and therapeutic vaccines designed to improve chemotherapy. While there is no doubt that progress is still being made, there are limitations to our animal model screening processes, which are further amplified by the lack of understanding of the immunological responses involved and the precise type of long-lived immunity that new vaccines need to induce. The challenge ahead is to optimize the planning for advanced clinical trials in poor endemic settings, which could be greatly facilitated by identifying correlates of protection.

**KEYWORDS:** animal models • BCG-replacement strategies • clinical development • mucosal delivery route • regulatory aspects • tuberculosis vaccines

The intimate relationship between Mycobacterium tuberculosis & the human host

**Human reservoir of M. tuberculosis**

Human tuberculosis (TB) is mainly caused by Mycobacterium tuberculosis (MTB) and to a lesser extent by Mycobacterium africanum, which are members of the M. tuberculosis complex (MTBC). These are obligate human pathogens with limited survival outside of the human body and no known animal reservoir. In addition to these human-adapted pathogens, MTBC includes various animal-adapted species, such as the bovine pathogen Mycobacterium bovis that can occasionally cause TB in humans. Although MTBC members differ in terms of host tropism and pathogenicity, there exists a strong evidence for a clonal population structure of MTBC, without signs of ongoing horizontal gene transfer. Accordingly, MTBC is a group of phylogenetically related members characterized by 99.9% similarity at the nucleotide level and identical 16S rRNA sequences [1].

**Global distribution of clinical isolates**

The acquired cellular response, as represented largely by CD4+ T cells, provides protective immunity and contributes to establish a latent infection, while also promotes the development of pulmonary lesions and the caseous necrosis required for transmission [5,6]. Since MTBC interacts with humans through antigen-specific CD4+ or CD8+ T cells, we would expect that in order to avoid immune recognition by the host, T-cell antigens were
among the most diverse genes in the MTBC genomes. However, human T-cell epitopes of MTBC are evolutionarily hyper-conserved and do not reflect any ongoing evolutionary arms race to afford immune evasion [7]. On the basis of hyperconservation of T-cell antigens, MTBC members would benefit from being recognized by the immune system of the host since the immune responses elicited by T-cell epitopes might in fact be more beneficial to the bacteria than to the host. The ensuing host immune responses contribute to tissue destruction and the formation of cavities in the host lung, which ultimately enhances transmission. In the context of host–pathogen co-evolution and supporting the ecological theory, contacts exposed to ‘modern’ MTBC were more likely to develop active TB compared with individuals exposed to ‘ancient’ MTBC. Additionally, it has been recently found that genetically diverse strains of MTBC vary widely in induction of an early inflammatory response during infection of human macrophages, with a significantly lower response to evolutionarily ‘modern’ lineages as compared with ‘ancient’ lineages [8].

Susceptibility of the host: human genetics
From a population infected with MTBC, only 5–10% of infected individuals will develop disease during their lifetime. Variation in susceptibility to TB can be attributed to environmental factors such as malnutrition but a portion is thought to be due to host genetic factors [9]. The early use of BCG was marked by a tragic accident in Lubeck (Germany) between 1926 and 1930. Infants received a batch of BCG vaccine contaminated with virulent MTB but not all developed tuberculosis [10]. Another evidence comes from twin studies showing that the rate of TB in monozygotic twins is twice that observed among dizygotic twins (60 and 35%, respectively) [11]. There are inter-individual variations in susceptibility to TB with genetic determinants contributing to the immune response to MTB infection. It has been identified multiple rare single-gene mutations linked with Mendelian susceptibility to mycobacterial disease [12,13]. Some studies have identified key immunological pathways involved in protective immunity against TB, such as the IL-12/23 and IFN-γ [13]. Quest for the genetic determinants of susceptibility to TB at the population level has so far been primarily driven by case–control studies of candidate genes. Studies based on such approaches identified over 20 genes genetically associated with susceptibility to pulmonary TB [12]. Recently, a genome-wide approach identified a number of loci that are promising candidate genes for susceptibility to pulmonary TB [9].

The inconsistent efficacy of BCG
Different sub-strains of BCG, different phenotypes
BCG which is currently the only licensed vaccine against TB, was developed between 1908 and 1921 by repeated sub-culture of an M. bovis strain isolated from a cow. A total of 231 passages resulted in attenuation of the strain. This was observed first in calves and subsequently in guinea pigs and other animal models [10]. In 1921, BCG was administered for the first time orally to a newborn and by late 1920s, the original BCG Pasteur was disseminated throughout the world.

Prior to the adoption of freeze-drying in the 1960s, individual laboratories preserved their strain by repeated sub-culture passages, and this resulted in appearance of different BCG sub-strains that became designated by the laboratory. Five main strains are used today in international immunization programs: Pasteur 1173 P2, Danish 1331, Glaxo 107, Tokyo 172-1 and the Russian BCG-I; the Moreau RDJ strain is used mainly in Brazil [14]. Genomic analysis of BCG strains has documented multiple molecular changes [15–17]. The main reason for BCG attenuation is the loss of the region of difference 1 (RD1) associated with loss of the immunodominant virulence factor the early secretory antigen of 6 kDa (ESAT-6). Multiple other deletions probably contribute to phenotypic differences between BCG strains and while there are clear reactogenicity differences, it is not clear whether strain differences are a significant factor contributing to the variable efficacy of BCG observed in clinic [18].

Dosage & methods of administration of BCG
Since 1974, intradermal BCG vaccination at birth has been included in the WHO Expanded Programme on Immunization (EPI) resulting in more than 3 billion cumulative vaccinations worldwide and approximately 100 million vaccinations per year [14]. The concentration of live particles in the vaccines ranges from 50,000 to 3 million per dose depending on the BCG strain [19].

BCG in developed & developing countries
BCG is effective against rare forms of severe childhood TB meningitis and miliary disease, however, the variation in protection against common pulmonary TB that BCG offers has generally been disappointing in trials conducted in the developing world [20]. In order to develop new vaccines against TB, we need to understand the possible factors and the mechanisms that could affect the efficacy of BCG in developed versus developing and underdeveloped countries. Such factors should be taken into consideration in design of clinical trials, as well as in preclinical studies, which should optimally mimic as closely as possible the conditions in the clinical setting where the new candidate is aimed for evaluation, taking into account the potential impact of poor socio-economic lifestyle, environmental mycobacteria and co-infection with other pathogens on the immune status of an individual.

Environmental mycobacteria
Saprophytic environmental species of non-tuberculous mycobacteria (NTM) are known to be common in soil and untreated water. In developing countries most people are skin test positive to many mycobacterial antigens, whereas in developed countries at higher latitudes this is less usual [22]. NTM are thought to affect BCG efficacy in pre-exposed children by masking or blocking ability of BCG to confer protective responses [23]. Continuing NTM exposure of individuals could
induce sensitization, as suggested by animal studies in which mice exposed to NTM need higher doses to shift from latent to progressive disease [24,25].

**Socio-economic conditions, co-infection with other pathogens & malnutrition**

The impact of socio-economic status on TB susceptibility and incidence is considered to be of prodigious significance [26,27]. Recent studies show that crowded, poorly ventilated, moist and dark places, where sunlight is limited are accounted for the high incidence of TB in the poor parts of developing countries, such as India [28]. Latent TB and low-socio-economic status have been shown to be predictors of TB disease in Worcester, a poverty stricken region near Cape Town, South Africa [29].

In the context of poor socio-economic conditions in developing countries, where BCG presents the greatest variability in protection as compared with developed countries, chronic viral (HIV) and parasitic infections (helminths, malaria) can greatly influence the population's susceptibility to TB and response to BCG [30,31]. Intestinal parasites, such as helminths, cause chronic immune system activation and associated immunosuppression [32], which can be a risk factor for increased TB susceptibility [33,34]. Infection of mothers with schistosomiasis and filariasis has been shown to influence infant responses to neonatal BCG immunization [35]. In mice, *Schistosoma mansoni* infection reduces the protective efficacy of BCG vaccination against virulent MTB [36].

Malnutrition is a child of poverty. Protein energy malnutrition has been shown to affect safety of BCG in mice [37] and impair development of cell-mediated immunity to BCG in mice [38] and in guinea pigs [39], where BCG has an established safety and efficacy profile, and in clinic it has been directly associated with increased risks of TB disease [40]. Protein deficiency is associated with tuberculin anergy (false-negative TST), which results in poor detection of clinical TB [41,42]. Malnutrition and helminths infection in children cause indeterminate results to the QuantiFERON-TB Gold In-Tube assay (QFT-IT) [43].

There is increasing evidence highlighting the importance of vitamin D as a potent modulator of human immune responses and in the control of MTB infection [44–46]. Vitamin D deficiency is directly linked to TB susceptibility [47] and clinical studies show that vitamin D supplementation accelerates resolution of inflammatory responses and enhances anti-mycobacterial immunity during TB treatment [48–50]. More recently, vitamin C was shown to have potent bactericidal effect on MTB, as high doses of vitamin C killed multi- and totally drug resistant strains, and the bacteria did not develop resistance to the vitamin [51].

A proposed model that might explain the variable BCG response could involve epigenetic mechanisms known to be modulated by environmental factors (e.g., nutrition, stress, pollution, infections and other environmental factors), which in turn can affect changes in immune system phenotype [52]. In addition to epigenetic modifications, action of microRNA on epigenetic markers has also been suggested to play a role in regulation and development of protective inflammatory immune responses to etiological agents [53] and could possibly affect regulation of immune response development to vaccination against these agents. Recent observations suggesting that vitamin D influences epigenetics provide a new insight for the importance of vitamin D in utero in reducing risk of chronic diseases later in life [54]. Emerging evidence suggests that chronic infections of mothers, stress and malnutrition can affect in utero development through alterations in postnatal gene expression and metabolic pathways central to accurate functioning and maintenance of health [55]. Although evidence exists for other inflammatory diseases, research is still required to understand how epigenetics could play a role in response to vaccination against TB and disease development later on in life. Recent observations show that intestinal microbiota in infants and adults may have implications in the development of immune responses against microbial infections [56–58]. Moreover, prenatal influences including stress, illness, diet and drug intake have been suggested to play a role in development of gut microbiota in neonates [57]. These data make one reason that chronic parasitic and viral infections, such as helminths and HIV in pregnant mothers as well as malnutrition, may have important implications in the development of the immune status of an infant. Epigenetics and gut microbiota could hold a key to the variable responses to BCG vaccination or to new vaccines (BCG-replacements or boosts) in the underdeveloped and developing world as compared with developed countries. It might be interesting to explore the role of poverty-associated factors in epigenetic mechanisms and gut microbiota in the development of immune responses following vaccination.

**Recent developments in clinical trials in the current global tuberculosis vaccine portfolio**

**Rational approach to selection & development of TB vaccine candidates**

Given the pipeline of TB vaccines in the three different stages of development (discovery, preclinical and clinical studies), a rational process for selection of TB vaccine candidates has been established in a joint effort between the two main initiatives in TB vaccine research in the world: the European TuBerculosis Vaccine Initiative (TBVI) and Aeras TB Vaccine Foundation, originated in the USA [59,60]. The development process of vaccine candidate is based on a stage-gating approach, which follows a linear pathway to determine if there is sufficient and robust evidence to allow further development of a specific product [59]. There are six stages that each vaccine candidate needs to go through to reach marketing authorization and these include: discovery, preclinical development, Phase I and Phase II/IIb for proof-of-concept, Phase III clinical trials, marketing application and market (licensure). Decision to proceed to the next stage occurs at a gate between each stage, using multiple criteria in the selection process. The evaluation criteria for advancing past each gate are defined according to the candidate’s characteristics, which divide into product quality, safety,
Annual Report 2012

infection without causing disease. As presented in the TBVI approach to human vaccinology, expected to mimic natural MTB from human origin are considered a classical Pasteurian cacy of BCG by insertion of other genes. Rationally attenuated BCG-replacement vaccines, namely recombinant BCG (rBCG) and live-attenuated BCG-replacement strategies divide into two classes of live vaccines: therapeutic candidates that have reached clinical trials conduct. In the face of multiple similar products a matrix pathway is used where head-to-head comparison within agreed animal model systems can help decision-making as can robust critical assessment of manufacturing, regulatory issues and intended TPP. As explained in the blue print, the criteria for the first three gates are well established (up to entry into early Phase II trials), but product-specific specifications for gates 4 through 6 are still under development at this time. TBVI and Aeras are currently working with vaccine developers and expert advisors on defining the rest of the stage-gating criteria crucial for down-selection of candidates as they progress to advanced clinical development (large-scale trials and manufacture), where resource availability and clinical trial sites become more limited. Clinical experience with the current vaccines would help in the definition of such down-selection criteria.

The current global TB vaccine portfolio consists of three main types of vaccine strategies, which are either preventive or therapeutic [23,61]. The preventive strategies embrace the priming BCG-replacement vaccines and subunit BCG boosts (or enhancers). Therapeutic candidates that have reached clinical development to date comprise inactivated forms of mycobacteria. In continuation, the most recent developments in TB vaccines are discussed, focusing on the candidates in clinical development.

**BCG-replacement strategies**

BCG-replacement strategies divide into two classes of live vaccines, namely recombinant BCG (rBCG) and live-attenuated MTB [23]. The rBCG candidates are designed to improve efficacy of BCG by insertion of other genes. Rationally attenuated MTB from human origin are considered a classical Pasteurian approach to human vaccinology, expected to mimic natural infection without causing disease. As presented in the TBVI Annual Report 2012 [201], today there are only two priming vaccines in clinical development, rBCG VPM1002 and the live-attenuated MTBVAC (Table 1).

VPM1002 (BCGΔureC::hly HmR) is an rBCG Prague with deleted urease C gene to allow optimal pH for the expression of biologically active lysteriolysin O (LLO) from *Listeria monocytogenes* and containing hygromycin resistance (HmR) gene [62]. LLO is expected to perforate the phagosome to allow escape of BCG into the cytosol potentially increasing the amount of bacterial-derived antigen available for presentation to CD8+ T cells through the cytosolic scavenger pathways. VPM1002 has successfully been evaluated in two Phase I clinical trials for safety and immunogenicity in healthy BCG-naïve and BCG-immune adults in Germany [63] and in South Africa [202]. The trial results showed that VMP1002 is safe and well tolerated and supported entry in a Phase IIa trial in healthy newborns in South Africa in 2012 [203]. Absence of severe adverse events after VPM1002 vaccination is of particular note since in the Phase I clinical trial of the rBCG Aeras-422, some study participants had reactivation of shingles [64]. In addition to some MTB antigens, including Ag85A, Ag85B and Rv4307, Aeras-422 expresses the perfringolysin O, which possesses similar membrane-perforating activity but is devoid of the inbuilt safety mechanisms characteristic of LLO [65]. It is worth commenting that the preclinical development plan (and the regulatory opinion) has been established with VPM1002 containing the Hyg antibiotic marker. For advanced clinical development, the current state of play in cGMP manufacture of markerless strain is important and bridging studies with the final vaccine construct without the antibiotic marker will have to be considered.

MTBVAC is live rationally attenuated MTB derived from the clinical isolate MT103 belonging to the widespread Euro-American Lineage. MTBVAC contains two independent stable deletion mutations in the virulence genes phoP and fadD26 without antibiotic resistance markers [66]. PhoP controls 2% of the genome of MTB including production of immunomodulatory cell-wall lipids and secretion of ESAT-6 so that phoP mutants can produce ESAT-6 but are unable to export it [68]. Deletion of fadD26 leads to complete abolishment of synthesis of the virulence lipids phthiocerol dimycocerosates (DIM) part of the lipid capsule. Rigorous preclinical characterization studies provide strong evidence that MTBVAC is at least as safe as, immunogenic and effective against MTB challenge as compared with BCG. In October 2012, MTBVAC received approval to enter first-in-human dose-escalation Phase I clinical trial for safety and immunogenicity in healthy adult volunteers in Lau-
sanne, Switzerland [204]. Three doses of MTBVAC are currently tested compared with standard human dose of BCG.

**BCG boosting strategies in clinical trials**

The global pipeline currently contains 9 subunit TB vaccine candidates in clinical trials (Table 1), which are mainly aimed for use as heterologous boosts in BCG-primed individuals, where BCG is given at birth and then boosting is applied with antigens specific for MTB (but which can also be expressed in BCG). Heterologous prime-boost immunization is considered to be highly effective for enhancing humoral and cellular immune responses [69] and reviewed by Dalmia and Ramsay [70]. There are also boosting strategies in the vaccine portfolio (as explained below), which are heterologous in terms of platform or delivery technology, but homologous in terms of antigen set. Subunit vaccines are based on one or a few MTB-specific
protein antigens using viral vectors or adjuvants as delivery system [23,64]. At present, only a small number of well-known MTB-specific protein antigens (some of which are expressed in BCG) are commonly employed in the construction of different TB vaccine candidates (subunits or rBCG). These antigens include the mycolyltransferase antigens 85A (Ag85A) and Ag85B, ESAT-6 or the low molecular weight protein antigen 7 (10 kDa) ExsH (TB10.4), which is used instead of ESAT-6 in some candidates to avoid cross-reactivity with the ESAT-6-based interferon gamma release assays (IGRA) (QFT-IT) used for clinical diagnosis of TB infection. Other proteins used in specific candidates include immunodominant Mtb32A (Rv1196) and Mtb39A (Rv0125) in M72 [71]. The latency-associated antigen Rv2660c in H56 fusion protein [72,73] or Rv1813, Rv2608, Rv3619 and Rv3620 in ID93 [74].

The delivery systems employed in current subunit vaccines comprise replication-deficient viral vectors or adjuvants [62,3]. The viral vectors include modified vaccinia Ankara (MVA), or adenovirus subtypes of human origin (AdHu). More recently, a modified simian adenoviral vector (chAdOx1), selected for low seroprevalence [75] and the Fowlpox virus, suggested to improve boosting of antigen-specific CD8+ T cells by malaria vaccine trials [76], have been employed in some TB vaccine subunit regimens as described in continuation. Adenovirus vectors are known for their natural tropism for the respiratory epithelium with ability to induce potent Th1-type responses and in recent works shown to have ability to differentially imprint innate cells at the mucosal site of immunization [77,78]. The main advantage of MVA vector is its satisfactory safety profile in HIV-infected individuals and its ability to induce polyfunctional, durable CD4 responses [79,80]. Adjuvant systems used for optimal delivery of subunit TB vaccines are mainly proprietary to the candidate and are not yet licensed. IC31® developed by Crucell has been used with several TB subunit candidates in clinical trials (Table 1). This adjuvant is a combination of a single-stranded oligodeoxynucleotide and an immunopotentiating peptide and serves as a depot formulation for slow antigen release [81].

**Boots delivered by viral vectors**

MVA85A employs MVA to deliver Ag85A. To date, MVA85A has been evaluated by different routes of administration (e.g., intradermal, intramuscular) in an array of more than 20 different Phase I and Phase II clinical trials for safety and immunogenicity in different target age group populations ranging from healthy adults, adolescents, children and infants to HIV-positive adults with or without latent MTB infection in the UK and in high-burden African countries [79,80,82-91]. All these trials show that MVA85A is safe and immunogenic with ability to induce robust, antigen-specific polyfunctional and durable CD4+ T-cell responses thought to be important for protection. In line with these results, the South African Tuberculosis Vaccine Initiative (SATVI) recently evaluated intradermal MVA85A in a Phase IIb trial for safety and efficacy in healthy BCG-vaccinated infants (6 months old) in Worcester [92]. MVA85A was safe and well tolerated but there was no further improvement of BCG efficacy following intradermal MVA85A vaccination. After 2 years of follow-up, 39 (2.8%) of 1395 infants in the placebo group and 32 (2.3%) of 1399 infants in the MVA85A group satisfied the primary definition of active TB disease. MVA85A is currently ongoing a protective efficacy study against TB disease in HIV-infected adults [209] as well as a Phase IIa safety and immunogenicity in infants born to HIV-positive mothers where MVA85A is given at birth, and not as a booster [93]. More recently, MVA85A entered safety evaluation in combination with other viral-
vectored vaccines in clinical development [206], as well as with the new chimp adenovirus ChAdOx1 85A [207] or the Fowlpox virus FP85 [76,268] both expressing Ag85A as in MVA85A with the idea to optimize immunogenicity and induce protection [94].

Ad5Ag85A is a recombinant human-type AdHu5 used to express Ag85A as in MVA85A [670]. Although, AdHu5 is considered one of the most robust gene transfer vehicles for in vivo T-cell activation, underlying seroprevalence of AdHu5 humoral immunity in humans may pose risk for interfering with TB vaccines of this kind (reviewed by Rowland and McShane [23]). The results of the recently completed Phase I study for safety and immunogenicity of a single intramuscular dose of Ad5Ag85A in BCG-immunized and in BCG-naïve healthy adult volunteers in Canada were recently presented at the Tuberculosis Vaccines 3rd Global Forum in Cape Town, South Africa (25–27 March 2013) [209,210]. The vaccine was safe, well tolerated and immunogenic with more potent T-cell immunity induced in BCG-vaccinated subjects. Pre-immunization anti-AdHu5 humoral immunity was found in most subjects but did not significantly dampen the potency of the candidate. Data support further clinical development of this candidate.

Ad35/AERAS-402 uses human Ad35, which was selected for low-level seroprevalence [96] to express a fusion protein of the three common antigens Ag85A, Ag85B and TB10.4 [97]. To date, the candidate is being developed for use in BCG-vaccinated individuals by the intramuscular route. In healthy BCG-vaccinated adults in South Africa and in the USA, Ad35/Aeras 402 was well tolerated, safe and induced Ag85A/Ag85B and TB10.4-specific immune responses [98]. The candidate has completed a Phase IIa trial for safety and immunogenicity in HIV-infected, BCG-vaccinated adults in South Africa [211], and is currently in a multi-center (Kenya, South Africa and Mozambique) Phase II dose-finding study for safety and immunogenicity in healthy BCG-vaccinated HIV-negative infants [212]. At the Tuberculosis Vaccines Third Global Forum 2013, data from South Africa were presented showing the candidate is safe and immunogenic and double administration of the highest dose may be the optimal vaccination strategy, as it induced persistent specific polyfunctional CD4+ T cells thought to be important in TB immunity [213].

**Boosts delivered by adjuvants**

M72/AS01E is a recombinant fusion protein of the immunostimulatory MTB-specific antigens Mtb32A (RV1196) and Mtb39A (Rv0125) in combination with adjuvant system AS01E [71]. AS01E is composed of two immunostimulants, 3-deacylated monophosphoryl lipid A (MPL) and QS-21, a detergent purified from the bark of Quillaja saaponaria, and a liposomal preparation. As presented at the Tuberculosis Vaccines Third Global Forum, in several Phase I and Phase II trials for safety and immunogenicity in PPD-negative, PPD-positive and HIV-positive adults in different TB-endemic countries, intramuscular M72/AS01E has demonstrated safety and tolerability [214]. In both TB-infected and -uninfected adults, the vaccine induces strong and persistent M72-specific CD4+ T cell and antibody responses [71,99]. M72/AS01E is currently enrolled in the largest multi-country, Phase IIb clinical trial for safety and efficacy, designed to administer two doses of M72/AS01E or placebo to HIV-negative adults over a 3-year follow-up period [214]. The study plans to enroll 7000 participants powered at 80% for a true vaccine efficacy of 70%, thus requiring 28 pulmonary TB events as confirmed by GeneXpert MTB/RIF or TB culture. India and sub-Saharan countries were selected to provide geographical diversity, trial capacity and TB incidence rate of 3/1000 persons per year in their HIV-negative populations.

Hybrid 1(H1) is a recombinant fusion protein of Ag85B and ESAT-6 initially designed to prevent acute TB disease [72] and developed to prevent reactivation of existing latent infection in adolescents or adults as well [215]. This candidate is currently being evaluated in early clinical trials formulated in two different adjuvants, IC31 (Intercell) or CAF01 [214]. Intramuscular H1-IC31 has successfully completed several Phase I trials in BCG-naïve and BCG-immune adults with latent TB infection (LTBI) [215]. Despite initial concerns with the transient false-positive responses developed to ESAT-6/CFP-10 QuantiFERON®-TB Gold (Cellestis) diagnostic test, development of the candidate has not halted and the vaccine is currently in Phase II clinical trials including HIV-infected individuals [215].

H1-CAF01 was recently evaluated in a Phase I trial to determine the safety of the CAF01 adjuvant in healthy adult volunteers, comparing Hybrid 1 alone with Hybrid 1 with three escalating CAF01 dose levels [216]. CAF01 consists of the immune-stimulating synthetic cord factor from MTB glycolipid trehalose-dibehenate (TDB), as TLR-independent immunomodulator, incorporated into cationic surfactant dimethyldioctadecylammonium bromide (DDA) liposomes offering a depot formulation for slow release [100].

H56-IC31: H56 is another recombinant protein, which in addition to early-secreted Ag85B and ESAT-6 (comprising H1) also contains the latency-associated antigen Rv2660 [72,101]. H56 is delivered in IC31 by the intramuscular route and like H1 it is aimed to prevent acute TB disease as well as reactivation of existing LTBI in adolescents or adults [215]. The candidate is in a Phase I safety and immunogenicity trial in adults (healthy and with LTBI) in South Africa and plans to start recruitment for a Phase I/IIa safety and immunogenicity of multiple dosage levels and dosing regimens of H56-IC31 in HIV-negative adults with and without latent infection in late 2013 are underway [215,217].

Hyvac 4-IC31 is a recombinant fusion protein of Ag85B and TB10.4 [102] formulated in IC31 and intended as a subunit boost to an existing BCG-induced immunity in infants and children [215]. Intramuscular H4-IC31 has undergone four Phase I safety trials in healthy BCG-immune adults, three of which were for adjuvant and antigen dose definition and one for safety and immunogenicity following recent BCG immunization in adults [215,218]. Aeras is preparing for participant...
Therapeutic vaccine candidates

Candidates that have reached clinical development as potential therapeutic strategies aim to reduce duration of the TB treatment [103] and to date they comprise inactivated forms of mycobacteria (MTB or NTM).

RUTI consists of liposomes containing detoxified fragments of heat-inactivated virulent MTB, grown under stress conditions and has been successfully evaluated in two clinical trials: a Phase I safety, tolerability and immunogenicity for dose definition in healthy adults in Spain [104], and in Phase II trial for safety, tolerability and immunogenicity following 1 month of DOTS treatment in subjects with latent TB infection in South Africa [221]. Archivel Farma is currently looking for a financial partner to test a single dose of RUTI in a Phase III trials as an adjunct to chemotherapy against active TB disease in people with LTBI; and second, to prevent relapse episodes in active TB patients by administering the vaccine in the continuation phase of treatment [209]. No clinical trials with RUTI are currently ongoing.

Mycobacterium vaccae is an inactivated whole cell M. vaccae (NTM). Initially, agar-grown inactivated M. vaccae was evaluated as a therapeutic vaccine in HIV-positive and in HIV-negative, MTB sputum-positive populations in different countries [105–109]. After the first Phase III efficacy trial of M. vaccae (the DarDar Trial) in which a 5-dose regimen was safe, immunogenic and effective in preventing TB in HIV-infected adults with prior BCG immunization [105], development of inactivated M. vaccae has shifted its TPP to a prophylactic strategy [222]. M. vaccae (DAR-901) is a broth-grown heat-inactivated polyantigenic M. vaccae manufactured by Aeras and is targeted as a TB prophylactic 3-dose series suitable for both HIV-infected and non-infected adults who have previously received BCG [222]. Non-clinical toxicology and immunogenicity studies have been initiated by Aeras to support entry into clinical evaluation for safety and immunogenicity in the USA and Tanzania in 2013.

Mw is a whole-cell heat-inactivated saprophytic non-pathogenic Mycobacterium indicus pranii, initially coded as Mycobacterium w [110], with an established immunotherapeutic role used beneficially in the treatment of leprosy [111,112]. Mw has also shown promising immunotherapeutic and immunoprophylactic potential against pulmonary TB disease in clinic [113,114]. Sputum conversion has been reported to appear more rapidly in patients who received DOTS + Mw as compared with DOTS alone [114,115]. The Drug Controller of India has already licensed Mw for use in humans [209]. A large-scale multi-centric Phase III evaluation sponsored by the Department of Biotech (DBT) (Government of India) and Cadila Pharmaceuticals to assess the immunotherapeutic effect of Mw as an adjunct to first-line antimicrobial therapy in pulmonary TB retreatment patients (category I and category II TB patients, and individuals with TB pericarditis) has recently been completed. Results have yet to be published (Table 1) [201,209,223].

Predoctoral animal models

Current animal models for selection of new vaccine candidates in the pipeline

The current animal models most used for vaccine evaluation are mouse, guinea pig and non-human primates (NHP) (macaques) [116,117]. SCID mice, which are highly immunocompromised as they lack T- and B-lymphocyte, have emerged as the reference model for safety consented among TB vaccine researchers and regulatory bodies [116,117]. When it comes to protective efficacy evaluation, there are many models but all present limitations [118]. The mouse model is the most readily used as it is cheaper and murine immunological reagents for TB assessment are readily available. Nevertheless, differently to human TB, commonly used mice strains (C57BL/6 and BALB/c) are highly resistant to MTB infection and do not form caseous granuloma in lungs. Moreover, human and mouse macrophages have been shown to use distinct mechanisms to kill intracellular MTB through mammalian Toll-like receptors (TLR) [119]. Moreover, human macrophages use a vitamin D-dependent mechanism to produce antimicrobial peptides not found in mice [120]. Unlike the widely employed mouse models, guinea pigs are highly susceptible to TB infection and as such are considered a more stringent model to evaluate new TB vaccines, which usually show promising results in mice [121,122]. Histopathology of guinea pigs is more similar to human TB disease, with formation of characteristic caseous granuloma. However, availability of guinea pig immunological reagents is quite limited and this animal model is mainly employed for protective efficacy evaluation of new TB vaccines. Macaque models have quite similar course of disease pathology to humans, and events of latency and reactivation have been
described [123]; their use is highly limited. Normally, NHP are used with advanced candidates close to clinical trials [117]. Other animals such as rabbits, cows, pigs or mini pigs are also explored as models [118]. Lack of standardized vaccine evaluation methods present a real challenge in the development of new TB vaccine candidates [60,124].

**Protective efficacy readouts used in preclinical studies**

Although different readouts, such as weight gain, survival or lung histopathology, are used to compare vaccine protective efficacy, the parameter most utilized is reduction of replication of MTB usually following aerosol challenge in vaccinated animals. Significant higher reduction of bacterial burden in lungs (>0.5 logs) induced by new candidates in comparison with BCG is a criterion currently used for new TB candidates to move to clinical development [116,117]. Nevertheless, considering that BCG is able to significantly reduce MTB challenge replication when compared with unvaccinated animals, we should reckon how predictable protective efficacy of >0.5 logs afforded by new candidates is for human TB disease in clinic (compared with BCG) [125].

**New models for TB transmission & latency**

Eradication of TB in 2050 could be possible, if new vaccines target prevention of TB transmission [60,125]. Moreover, since TB is transmitted only during the active form of the disease, prevention of TB reactivation would have a dramatic impact on TB pandemia [60,125]. Nevertheless, animal models for transmission and reactivation are quite limited. In the case of mice, the model used for vaccines study, the different lung architecture and the absence of cavitation in infected lungs make difficult the establishment of a murine transmission model. Even though some works propose a transmission model in mouse [126], it is not clear whether this transmission occurs aerogenically. Natural infection of guinea pigs exposed to air ventilated from hospital TB wards has been tested [126]. Efforts are currently underway to re-establish this model for evaluating vaccines and immune responses that may prevent transmission [224].

Regarding models for latency and reactivation, the cynomolgus macaque model results highly advantageous with respect to other animals. Following low-dose aerosol challenge, this model presents human-like episodes of TB disease: primary disease, latent infection and reactivation. Previous studies show that cohorts of macaques infected with a low-dose challenge demonstrated presence of infection (tuberculin skin test positivity), but only a part of the animals developed active TB [123,127]. This model has been expanded to include PET/CT imaging allowing visualization of the infection dynamics during different stages of infection [225].

**Biomarkers in preclinical & clinical setting**

Development of an improved vaccine against TB is hindered by the lack of a surrogate of protection. Efficacy of new TB vaccines in humans can only be evaluated by expensive and time-consuming efficacy trials within TB endemic areas [123]. In experimental animal models of TB vaccination, IFN-γ-producing CD4+ Th1 cells have been a popular demonstration of vaccine immunogenicity as measured. In humans these observations are being made on the circulating pool of lymphocytes present in the blood [623]. Nevertheless, it is becoming more evident that IFN-γ production by CD4+ T cells may be important for controlling onset of TB disease, but it is not a clear correlate of vaccine-induced protection [128]. In the search for better correlates of vaccine-induced production, light is being shone on the stronger correlation between polyfunctional Th1 cells and protection given to the host. However, whether multifunctional T cells are the best clinical readout to predict TB vaccine efficacy is now however under scrutiny, following a trial in South African infants showing no correlation of multifunctional T cells with TB protection when analyzed 10 weeks post-BCG vaccination [6,129].

It is critical that vaccines with the greatest potential to protect are selected for efficacy trials. Mycobacterial growth inhibition assays (MGIAs) have been developed with the hope to correlate data with protection in animals, which could aid in the selection of the best vaccine candidates [130]. More recently, a human challenge model using intradermal BCG administration in previously vaccinated individuals with BCG has been clinically tested with the idea to identify correlates of antimycobacterial immunity [131]. With a recently reported transcriptionic footprint specific for active disease in TB patients [132], transcriptional profiling is becoming more and more frequent as an additional immunological approach in clinical trials of new TB vaccines, where gene expression signatures are compared in samples from various time points [124]. More recently, as presented at the Third Global Forum for Tuberculosis Vaccines in Cape Town, a study in South African adolescents conducted at SATVI identified a gene signature of risk of disease differentially expressed between TB cases and controls [226]. It is postulated that correlates of risk in adolescents could allow target enrolment in vaccine trials and guide studies of vaccination-induced correlates of protection against TB disease [133]. With the current state of the art, only efficacy data from Phase III clinical trials of new vaccines will help correlate clinical immunological data with vaccine-induced protection.

**Rethinking vaccination route**

According to meta-analysis studies, the inconsistent efficacy of BCG is thought to be due to waning vaccine-induced immune responses with age which can reach an average of 14% overall efficacy after 10 years [134]. As a result, a consensus is emerging that new vaccine strategies should be targeted for prevention of TB in adolescents and adults where incidence is relatively high [133,135]. Nevertheless, epidemiological data in countries with routine use of BCG show that the highest incidence of TB cases in HIV-negative individuals is comparable early in life between 0 and 4 years of age and in adolescents and adults, and it is lowest between 5 and 14 years of age reaching nadir at about 10 years of age [136,137]. These data suggest that there appears to be a natural susceptibility to TB, which BCG is not
able to overcome regardless of age, and that it could be a question of quality rather than durability of the immune responses induced by BCG against the common form of the disease responsible for transmission.

One hypothesis for BCG inconsistent efficacy against MTB infection and disease is that circulating T-cell immunity induced upon vaccination (by intradermal route) is inadequate to reach the lungs on time when MTB infection occurs [138]. The natural route of MTB transmission is by aerosol and the nasal cavities are usually the first port of entry for the pathogen, while pulmonary mucosal tissues are the primary sites for establishment of infection [70]. The mucosal route of immunization has been suggested to have advantage over other routes to elicit protective immune responses in the lung, the site of primary TB infection [139]. Animal vaccination experiments by aerosol delivery of the BCG nanomicroparticle have shown significant reduction of bacterial burden in lung and spleen after MTB challenge as compared with parenteral BCG [140]. Moreover, in recent years, increasing number of new subunit strategies have been evaluated as heterologous boosts by the intranasal route to enhance efficacy of parentally primed BCG immune responses [70]. Ad5Ag85A, when administered intranasally but not intramuscularly, afforded better protection against M. tuberculosis aerosol challenge than cutaneous BCG and enhanced protection when given as a boost to BCG in both BALB/c mice and guinea pigs [95,141,142]. McMaster University, where the vaccine was conceptualized, plans to conduct a Phase I trial evaluating aerosol delivery of Ad5Ag85A [209]. Intranasal delivery of MVA85A significantly enhanced BCG-primed circulating immune responses and inhibited bacterial growth by up to 1.5 logs following aerosol MTB challenge in mice when compared with BCG alone [143]. Ad35/Aeras 402 showed efficacy against intranasal MTB challenge in two strains of mice by the intramuscular and intranasal routes [97]. Despite these promising data, the intranasal delivery of live vaccines carries serious safety risks inherent with this route (dissemination to the brain and potential lung disease in false-negative individuals with LTBI) and to date only the live influenza vaccine (Hib) is given intranasally in clinic. For future use of this route, extensive preclinical and clinical characterization with new TB vaccine boosts is imperative. In this context, the safety and immunogenicity of MVA85A delivered by the aerosol route to the lungs of macaques was recently published [144] and currently inhaled preparation of live MVA85A is currently being tested for safety and immunogenicity in a Phase I trial in healthy adults in the UK [227].

There are two trends in TB vaccine development that warrant mentioning. First, there is a new interest in BCG and novel TB vaccines for prevention of infection (and not just prevention of disease). This concept was introduced at the 3rd Global Forum on TB Vaccines in Cape Town, and Aeras in collaboration with SATVI are planning a prevention of infection trial with the H4 vaccine and BCG in adolescents. Second, in addition to the very important issue of considering alternative vaccination routes such as mucosal, there are developments in improving the administration technique of intradermal BCG, which may also have implications for future novel vaccines, particularly for the intradermal route. For example, an ongoing trial at the SATVI site (funded by PATH and WHO) is comparing BCG administration via conventional syringe and needle to a needle-free jet injector device [228]. Other devices to aid vaccine administration such as intradermal needle adaptors have also been developed for use in the field.

Experience with entry of the first-ever live MTB vaccine in clinical trials

MTBVAC is the first live-attenuated vaccine based on MTB to enter clinical trials. The construction and preclinical characterization of MTBVAC fulfills the Geneva consensus safety requirements for progressing live mycobacterial vaccines to Phase I clinical evaluation [116,117]. Two stable deletion mutations and safety that is at least comparable with BCG in the relevant animal models are the safety criteria for live TB vaccines. The Spanish biopharmaceutical company Biofabri produced and characterized MTBVAC as a freeze-dried preparation in compliance with cGMP following European Pharmacopoeia monograph and the WHO recommendations to assure the quality, safety and efficacy of BCG vaccines.

To date, the development of MTBVAC, which is based on the stage-gating approach described in the blueprint [60], has been accomplished with the help of external expert advisors appointed by and facilitated through the TBVI Product Development Team (TBVI PDT) and Clinical Development Team (TBVI CDT) [228]. Early contacts with relevant regulatory authorities were imperative for establishing a cGMP production process and design preclinical characterization studies to support conduct of first-in-human evaluation with the final product. Biofabri is currently optimizing a scale-up industrial process to support large-scale clinical trials of MTBVAC as a BCG-replacement strategy, given once at birth by the intradermal route. The next steps of MTBVAC development require safe age de-escalation in healthy newborns in high-incidence countries. This challenging and complex task requires effective resource mobilization and a robust package to convince relevant regulatory bodies for safety, immunogenicity and promising efficacy profile.

Lessons to be learned from the first efficacy clinical trial of MVA85A

Although the Phase IIb efficacy results with MVA85A in BCG-immunized infants were not as anticipated based on rigorous preclinical animal data [122,145,146], the trial represents a great milestone for clinical research in South Africa and for the global TB vaccine development community providing valuable information that can serve in the future development of vaccine candidates [92,147]. The SATVI team showed top of the line team expertise through this elegantly designed, organized and conducted in the most efficient manner possible trial showing the world that conducting high-quality research at the heart of the TB epidemic in a resource limited setting is possible [148].
In this trial, approximately 3% (39 of 1395) of the infants in the BCG control group satisfied the primary definition of active TB, with conversion in 12% of the infants in this group. The poor efficacy data in the BCG control group correlated with lack of antigen-specific Th1 and Th17 CD4+ T-cell responses. Th1 and Th17 antigen-specific T-cell responses were only modestly induced following vaccination with MVA85A and correlated with lack of improvement of protection in BCG-vaccinated infants. An earlier clinical trial in Gambian infants showed reduced immunogenicity of MVA85A when administered with EPI vaccines [87]. Another clinical trial in South African infants also investigated the role of age on MVA85A immunity showing that infants and children had lower pre-vaccination Ag85A-specific T-cell responses as compared with adolescents and adults [90]. However, significantly greater responsiveness in children and infants were observed at long term after MVA85A administration. These data suggest that progress will require frank appraisal of the gaps in our understanding of protective immunity and appropriate use and interpretation of the available animal models [124,149].

Expert commentary & five-year view

TB is transmitted by the respiratory route and role of mucosal immunity against MTB infection is becoming of increasing interest in the development of new TB vaccine strategies [150]. Future delivery of new vaccines by the inhaled or intranasal routes aiming at stimulating mucosal immunity in the lungs might induce the proper catalog of innate and cell-mediated immune responses that could virtually protect during the course of MTB infection [77]. However, potential risks of live vaccine dissemination to the brain or extensive lung inflammation inherent with respiratory routes require rigorous preclinical and clinical characterization. Given the consistent efficacy data with mucosal delivery of new subunit vaccines, the success of future boosting vaccination may lie in switching to mucosal delivery.

In addition to conferring protection against severe forms of childhood TB, observational studies have suggested that BCG may have non-specific beneficial effects on overall survival [150]. Recent studies showed that BCG vaccination results in training of innate immunity against non-mycobacterial infections and unrelated bacteria (Staphylococcus aureus) and fungi (Candida albicans) offering a potential immunological explanation for BCG’s non-specific effect on overall mortality [151]. Moreover, BCG vaccination has been associated with induction of potent Th1-type immunity at birth that can enhance immune responses to other neonatal vaccines [152].

Concerning the inconsistent efficacy of BCG against common forms of TB disease responsible for transmission, epidemiological data [21,134] provide evidence that a new BCG-replacement strategy effective against all forms of TB is necessary. Experience with currently utilized whole-cell vaccines indicates that using the human pathogen is the optimal approach for inducing efficient protection [153] and live-attenuated strategies are the best approach to confer durable T-cell immune responses [5]. New live-attenuated MTB could present a successful vaccination approach to replace BCG. In addition, the close genealogy of MTB and M. bovis suggests that live-attenuated MTB vaccines could share the live-saving benefits observed with BCG, but they should be addressed in preclinical and clinical studies. Also, a more effective intradermal vaccine at birth, capable of inducing adequate immune memory holds potential to effectively respond to subunit (potentially mucosal) regimens.

The current animal models are imperative for selection and progress of new TB vaccines to clinical development in the global pipeline. However, new models for TB transmission, latency and neonatal disease are required to allow better vaccine appraisal and help predict outcome of clinical trials. Moreover, new candidates should be able to offer efficacy against different clinical isolates of MTB. New BCG-replacement approaches should be inexorably tested in neonatal animal models not only to assess their safety profile in this population, but also to test their vaccine efficacy in an animal model with an immature immune system.

Innovative reliable models are needed to mimic as close as possible the real clinical setting and to be able to predict outcome of long and expensive efficacy trials. An ideal trial design requires a population with a high incidence of TB and a competent immune system where specific endpoints are easily determined [60]. However, countries with the highest TB burden are usually the poorest and face the problems of malnutrition, co-infection with other pathogens (helminths, malaria, HIV) and poor sanitation, which can severely impact on immune status and on the individual’s response to a potentially promising vaccine. The current models for screening utilize healthy specific pathogen-free animal models, where even BCG leaves little room for improvement. And efficacy data observed in these models are normally extrapolated to predict outcome of clinical trials in poor endemic areas. The use of novel animal models able to predict vaccine efficacy (or lack of efficacy) in such scenarios would be of major relevance in the design of future clinical trials in poor TB endemic settings.

Biomarkers in clinic are needed for prediction of vaccine efficacy against TB vaccine evaluation and to shorten costly efficacy trials [93]. For accelerating successful vaccine development, new human immunology-based clinical research initiatives need to be implemented with the goal of elucidating and more effectively generating vaccine-induced protective immune responses [154]. Regarding concerns with false-positive results to ESAT-6 IGRA assays in clinic, it appears that such data would not halt development of promising vaccine candidates. When planning new vaccine clinical trials, early regulatory contacts are of extreme importance in high-burden developing countries where the regulatory and ethics expertise required for approving TB vaccine trials is limited [155].

Very substantial efforts have been made over the past decade to develop vaccines against TB and R&D should continue to ensure that the robust pipeline continues to expand and strengthen. Since the global economic crisis has negatively...
impacted the funding of health research, it is critical that researchers and advocates unite to educate policy makers about the need for new TB vaccines and the role they can play in supporting TB vaccine research and clinical development in poor socio-economic conditions [135]. In the coming years, the first human efficacy data will be available from different viral-vectored vaccines expressing a limited set of antigens. Robust safety and immunogenicity data are imperative for conduct of future Phase IIb safety and efficacy trials of new BCG-replacement regimens such as MTBVAC and VPM1002 in neonates. Such trials are central to demonstrate whether these vaccines confer superior protection to BCG and surveillance of vaccinated neonates would demonstrate whether protection is long lasting.

Financial & competing interests disclosure
This work was supported by grant BIO2011-23555 from Spanish Ministry of Economy and Competitiveness and European Commission FP7-funded NEWTBVAC 241745 and European & Developing Countries Clinical Trials Partnership (EDCP, http://ec.europa.eu/-funded Collaboration and Integration of Tuberculosis Vaccine Trials in Europe and Africa (EDCTP-TBTEA). J Gonzalo-Asenio and C Martin are co-inventors on a composition of matter patent 'tuberculosis vaccine' filed by the University of Zaragoza. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Key issues

- Although the current animal models are essential for moving vaccine candidates to clinical trials, there is a growing awareness of their limitations for screening processes. New animal models for latency, transmission and neonatal safety and protection are necessary.
- It would be important to develop new vaccine candidates aimed at blocking infection and not just preventing disease.
- Biomarkers (immunological correlates of protection and risk of disease) are needed to predict vaccine efficacy to help progress between clinical trials and shorten duration of costly efficacy evaluation. Gene expression profiling in early clinical and in subsequent efficacy trials could help identify potential biomarkers for specific vaccine-induced protection.
- There appears to be a natural susceptibility to human tuberculosis, which Bacille Calmette–Guérin is not able to overcome. A more effective intradermal vaccine at birth, capable of inducing adequate immune memory holds potential to effectively respond to heterologous subunit regimens.
- Switching to the natural route of infection for vaccine delivery of heterologous boosts may be a key for success.
- Design of clinical trials should take into account impact of socio-economic conditions and co-infection with other pathogens/parasites, which could alter vaccine-induced immune responses and efficacy.

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• of interest
•• of considerable interest


•• A new scenario for the evolution of Mycobacterium tuberculosis complex suggesting that the common ancestor of the tubercle bacilli resembled M. tuberculosis or Mycobacterium canetti and could well have been a human pathogen already.


37 Efficacy study in mice showing reduction of BCG efficacy in pre-existing *Schistosoma mansoni* infection.
Recent developments in TB vaccines


- Recent study showing capacity of vitamin C to kill *Mycobacterium tuberculosis* (MTB) by the Fenton reaction.


- The first Phase I trial of rBCG VPM1002 showing safety and immunogenicity in BCG-naïve and BCG-vaccinated adults, which supported a Phase IIa safety and immunogenicity in healthy newborns in South Africa (NCT01479972).


- Construction and characterization of the first live-attenuated *M. tuberculosis* vaccine to enter first-in-human clinical evaluation.


- Clinical trial in South African adults showing the promising immunogenicity profile of M72/AS01 vaccine candidate. As a result, this candidate is enrolled in a multi-center Phase IIb efficacy trial (the largest to date).


- Results indicate that H56/IC31 boosting is able to control late-stage infection with *M. tuberculosis* and contain latent tuberculosis in cynomolgus macaques, providing a rationale for the clinical development of H56.


- MVA85A was evaluated in combination with Fowlpox virus (FP9) expressing Ag85A (FP85A) as in MVA85A with the aim to improve boosting of antigen-specific CD8+ T cells as suggested by malaria vaccine trials following vaccination with two heterologous recombinant poxvirus vectors.

77 Jeyanathan M, Damjanovic D, Shaler CR et al. Differentially imprinted innate immunity by mucosal boost vaccination determines antituberculosis immune protective outcomes, independent of T-cell

- This study reveals that vaccine vectors may differentially imprint innate cells at the mucosal site of immunization, which can impact immune-proTECTive outcome, independent of T-cell immunity, and it is of importance to determine both T-cell and innate cell immunity in vaccine studies.


- This study showed that coadministration of MVA85A with EPI vaccines reduces immunogenicity to this candidate in Gambian infants.


- MVA85A induced robust, polyfunctional, durable CD4 and CD8 T-cell responses in South African infants. These data supported efficacy evaluation of MVA85A in infants. This study also investigates the role of age on immunity showing that although infants and children had lower pre-vaccination Ag85A-specific T-cell responses as compared with adolescents and adults, significantly greater responsiveness in children and infants were observed at long term after MVA85A administration.


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- The second Geneva consensus for progressing clinical development of live TB vaccines beyond Phase I trials in TB-endemic countries.


- The first head-to-head comparison of 24 EU TB vaccine candidates inside the EU FP5-funded TB Vaccine Cluster Project, using BCG as the reference comparator. Single vaccination with the *phoP*-based vaccine SO2 and two boosting doses of MVA85A conferred significant efficacy and 100% survival as compared with 33% survival rate in the BCG group, following high-dose aerosol challenge with virulent MTB.


- This manuscript summarizes the consensus arrived at a meeting of South African and international stakeholders on specific late phase clinical trial design issues for tuberculosis vaccine efficacy in adolescents and adults.


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Recent developments in TB vaccines

Review

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206 A Phase I, open label trial to evaluate the safety and immunogenicity of AERAS-402 followed by MVA85A in BCG vaccinated adults (TB032).
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