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Safety and immunogenicity of candidate vaccine M72/AS01$_E$ in adolescents in a TB endemic setting

Adam Penn-Nicholson[a,*,1], Hennie Geldenhuys[a,1], Wivine Burny[b], Robbert van der Most[b], Cheryl L. Day[a,c,d], Erik Jongert[b], Philippe Moris[b], Mark Hatherill[a], Opokua Ofori-Anyinam[b,2], Willem Hanekom[a,2], the Vaccine Study Team,

Vaccine Study Team: Anne Bollaerts[b], Marie-Ange Demoitie[b], Angelique Kany Kany Luabeya[a], Evi De Ruymaeker[b], Michele Tameris[a], Didier Lapierre[b], Thomas J. Scriba[a]

[a] *South African Tuberculosis Vaccine Initiative, Institute of Infectious Disease and Molecular Medicine & Department of Paediatrics and Child Health, University of Cape Town, Cape Town, South Africa*
[b] *GSK Vaccines, Rixensart, Belgium*
[c] *Department of Global Health, Rollins School of Public Health, Atlanta, GA, USA*
[d] *Emory Vaccine Center, Emory University, Atlanta, GA, USA*

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ABSTRACT

Background: Vaccination that prevents tuberculosis (TB) disease, particularly in adolescents, would have the greatest impact on the global TB epidemic. Safety, reactogenicity and immunogenicity of the vaccine candidate M72/AS01$_E$ was evaluated in healthy, HIV-negative adolescents in a TB endemic region, regardless of *Mycobacterium tuberculosis* (*M.tb*) infection status.
Methods: In a phase II, double-blind randomized, controlled study (NCT00950612), two doses of M72/AS01$_E$ or placebo were administered intramuscularly, one month apart. Participants were followed-up post-vaccination, for 6 months. M72-specific immunogenicity was evaluated by intracellular cytokine staining analysis of T cells and NK cells by flow cytometry.
Results: No serious adverse events were recorded. M72/AS01$_E$ induced robust T cell and antibody responses, including antigen-dependent NK cell IFN-γ production. CD4 and CD8 T cell responses were sustained at 6 months post vaccination. Irrespective of *M.tb* infection status, vaccination induced a high frequency of M72-specific CD4 T cells expressing multiple combinations of Th1 cytokines, and low level IL-17. We observed rapid boosting of immune responses in *M.tb*-infected participants, suggesting natural infection acts as a prime to vaccination.
Conclusions: The clinically acceptable safety and immunogenicity profile of M72/AS01$_E$ in adolescents living in an area with high TB burden support the move to efficacy trials.

1. Introduction

Tuberculosis (TB) is the second leading cause of mortality worldwide due to a single infectious agent, leading to 9.0 million incident cases and 1.5 million deaths a year [1]. TB results in substantial personal, social, public health, and economic cost. South Africa faces a particularly high burden of TB disease, with the second highest annual incidence of TB cases in the world. Globally, the TB epidemic is compounded by the emergence of drug resistance; novel vaccination strategies may impact both drug sensitive and resistant disease.

Bacille Calmette-Guérin (BCG) is the only currently licensed vaccine against TB disease. Although BCG has been in use since 1921, it provides highly variable and mostly poor protection against pulmonary TB disease in adolescents and adults [1–5]. Adolescents and

* Corresponding author at: South African Tuberculosis Vaccine Initiative, University of Cape Town, Wernher Beit S2.01, Anzio Rd., Observatory, 7925 Cape Town, South Africa. Tel.: +27 21 404 7608; fax: +27 21 406 6693.
E-mail address: adam.penn-nicholson@uct.ac.za (A. Penn-Nicholson).
[1] These authors contributed equally to this work.
[2] Equal senior authorship.

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adults with TB disease spread *Mycobacterium tuberculosis* (*M.tb*), and should therefore be the main target populations of novel TB vaccination strategies [6].

Fourteen TB vaccine candidates are currently in clinical testing [7]. Most novel TB vaccine candidates aim to boost or modulate pre-existing T cell responses against *M.tb*. M72/AS01$_E$, one such vaccine candidate, is a recombinant fusion protein (M72) derived from Mtb32 and Mtb39, adjuvanted with AS01$_E$[8]. M72/AS01$_E$ has shown promise in multiple Phase I and IIa clinical trials in adults [9–14], including *M.tb*-infected persons living in a high TB burden setting. In these studies, the vaccine had a clinically acceptable safety profile and induces high magnitude M72-specific CD4 T cell responses, including a complex pattern of Th1 cytokines. However, the vaccine has not been evaluated in adolescents, a major target population for novel vaccination strategies. The aim of this study was to assess safety, reactogenicity and immunogenicity of two doses of M72/AS01$_E$ vaccination in healthy, Human Immunodeficiency Virus (HIV) uninfected adolescents living in a TB endemic setting.

2. Methods

2.1. Study design

This Phase II, double-blind randomized, controlled trial was approved by the University of Cape Town Health Sciences Human Research Ethics Committee (ClinicalTrials.gov, NCT00950612), and conducted in accordance with the Helsinki Declaration and Good Clinical Practices. Informed consent was obtained from the legal guardians and assent from the participants prior to screening.

2.2. Participants and vaccination

Greater detail of all procedures, including the vaccine, assays and statistical analysis, can be found in the supplementary material. Briefly, we aimed to enroll 60 adolescents aged 13–17 years from the Cape Town region of South Africa if they were healthy, HIV-negative, with no previous or current TB disease, and regardless of *M.tb* infection status (determined by QuantiFERON TB Gold In-Tube test (QFT)). Screening procedures included physical examination, chest X-ray, blood tests for hematology and biochemistry and a pregnancy test in females. Following blinded randomization, 40 participants were allocated to receive 2 doses of M72/AS01$_E$ (10 μg M72 adjuvanted with AS01$_E$, an adjuvant system containing 25 μg 3-O-desacyl-4'-monophosphoryl lipid A (MPL), 25 μg QS-21 Stimulon® [Quillaja saponaria Molina, fraction 21; licensed by GSK from Antigenics Inc., a wholly owned subsidiary of Agenus Inc., a Delaware, USA corporation] and liposome) and 20 to receive 2 doses of placebo (saline), on study days 0 and 30, administered intramuscularly.

2.3. Safety and reactogenicity evaluation

Injection site reactions, solicited and unsolicited systemic adverse events (AEs), and safety blood abnormalities were evaluated by diary card completion, physical examination and laboratory testing. Follow up clinic visits were performed 1 and 7 days after each vaccination, and on days 60 and 210 after the first vaccination.

2.4. Antibody ELISA

On study days 0, 30, 60 and 210, total anti-M72 IgG was measured in serially-diluted serum by ELISA, as previously described [10,14].

2.5. T cell intracellular cytokine staining assay

Two intracellular cytokine staining (ICS) assays were completed on samples collected on study days 0, 7, 30, 37, 60, and 210. First, whole blood was incubated with an M72 peptide pool, or with recombinant M72 fusion protein, as previously described [15,16]. Expression of IFN-γ, IL-2, TNF-α, IL-17, Ki67 and PD-1 was determined in CD4 and CD8 T cells. Second, isolated and stored PBMC were later thawed and incubated with the M72 peptide pool, as previously described [10,17]. Expression of CD40L, IFN-γ, IL-2 and TNF-α were determined in CD4 and CD8 T cells. Cells were acquired on a LSR II flow cytometer (BD Biosciences).

2.6. NK cell intracellular cytokine staining assay

$CD56^+CD16^{+/-}$ NK cell expression of IFN-γ and CD69 was measured following PBMC incubation with an M72 peptide pool, using an adapted ICS as previously described [18,19].

2.7. Data analysis

Frequency of AEs was described per number of administered doses, by type (injection site, systemic, laboratory), and by severity, seriousness and causality. Frequency and pattern of expression of different markers were outcomes of the ICS; data were analyzed using FlowJo software (TreeStar). Specific responses were calculated by subtraction of response frequencies in unstimulated samples from stimulated samples. Antibody results were described as geometric mean concentrations (GMC); a response was defined as >2.8 ELISA units/mL. Statistical comparisons between groups and time points were assessed with nonparametric tests, using GraphPad Prism 6.0d (GraphPad Software). Analysis were per protocol unless otherwise indicated.

3. Results

3.1. Participants

Sixty healthy, HIV-negative adolescents (median age 15.0 years, interquartile range – IQR – 14.1–16.3) were enrolled (Table 1). All participants had documented evidence of BCG vaccination or BCG scar. On Day 0 and Day 30, forty participants received M72/AS01$_E$ vaccine, and twenty received placebo. Demographic characteristics and reasons for exclusion did not differ between groups at baseline (Table 1 and Fig. S1).

3.2. M72/AS01$_E$ had a clinically acceptable safety profile

No participant experienced a serious adverse event (SAE) or withdrew due to an AE. AEs were reported in the 7 day post-vaccination period after 93.8% of all doses in the M72/AS01$_E$ group and after 57.9% of all doses in the placebo group (Table S1). In the M72/AS01$_E$ group, local AEs were reported after 90% of doses and general AEs after 75% of doses. In the placebo group, local AEs were reported after 26.3% of doses and general AEs after 44.7% of doses. 92.5% of M72/AS01$_E$ recipients had AEs after dose 1 and 95% after dose 2; these frequencies were 61.1% and 55% in placebo recipients, respectively.

The most common M72/AS01$_E$ associated local AE was pain, after 90% of doses, followed by swelling and redness, after 34% and 21% of doses, respectively (Table 2). Pain occurred after 21% of placebo doses, and swelling and redness each after 5% of doses.

Headache, fever, and fatigue were the most frequently reported systemic AEs among M72/AS01$_E$ recipients, after 54%, 45%, and 39% of doses, respectively, compared to 16%, 5%, 13% in the placebo group (Table 2).