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H1:IC31 vaccination is safe and induces long-lived TNF-α^+IL-2^+CD4 T cell responses in *M. tuberculosis* infected and uninfected adolescents: A randomized trial

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ABSTRACT

Background: Control of the tuberculosis epidemic requires a novel vaccine that is effective in preventing tuberculosis in adolescents, a key target population for vaccination against TB.
Methods: Healthy adolescents, stratified by *M. tuberculosis*-infection status, were enrolled into this observer-blinded phase II clinical trial of the protein-subunit vaccine candidate, H1:IC31, comprising a fusion protein (H1) of Ag85B and ESAT-6, formulated with the IC31 adjuvant. Local and systemic adverse events and induced T cell responses were measured after one or two administrations of either 15 μg or 50 μg of the H1 protein.
Results: Two hundred and forty participants were recruited and followed up for 224 days. No notable safety events were observed regardless of H1 dose or vaccination schedule. H1:IC31 vaccination induced antigen-specific CD4 T cells, co-expressing IFN-γ, TNF-α and/or IL-2. H1:IC31 vaccination of *M.tb*-uninfected individuals preferentially drove the emergence of Ag85B and ESAT-6 specific TNF-α^+IL-2^+CD4 T cells, while H1:IC31 vaccination of *M.tb*-infected individuals resulted in the expansion of Ag85B-specific but not ESAT-6–specific TNF-α^+IL-2^+CD4 T cells.
Conclusions: H1:IC31 was safe and immunogenic in uninfected and *M.tb*-infected adolescents. Two administrations of the 15 μg H1:IC31 dose induced the greatest magnitude immune response, and was considered optimal (South African National Clinical Trials Register, DoH-27-0612-3947; Pan African Clinical Trial Registry, PACTR201403000464306).

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1. Background

A core component of the global strategy to control tuberculosis (TB) is the development of efficacious vaccines that prevent infection and disease in adolescents and adults [1–4]. Bacille Calmette Guerin (BCG), the only licenced TB vaccine, has been widely administered for decades, but has been ineffective in controlling the worldwide epidemic [2]. Strategies involving a number of candidates are being assessed with heterologous vaccination of adolescents or adults to boost the immunity induced by either BCG, *Mycobacterium tuberculosis (M.tb)* or environmental mycobacterial infection thought most likely to have the greatest impact on the epidemic [1,3].

Adolescent and adult populations are at particular risk of TB disease. Adolescents typically manifest with adult-type pulmonary TB disease [5], which is primarily responsible for transmission of *Mycobacterium tuberculosis* (*M.tb*). Vaccination with a protective vaccine in adolescents could interrupt disease transmission in both the target population and the population at large [6]. In settings where TB is endemic, a large proportion of adolescents have already been exposed and/or infected with *M.tb* [7,8]. Prior *M.tb* or nontuberculous mycobacteria infection can interfere with vaccination, as described for live mycobacterial vaccines [9], or potentially compromise or enhance the antigen-specific response to vaccination [10,11]. Additionally, prior exposure may increase reactogenicity to vaccination due to a hypersensitivity reaction [3]. Vaccination of adolescents thus requires a preventive vaccine that is safe, immunogenic and efficacious in *M.tb* infected and uninfected persons.

H1 is a subunit fusion protein of *M.tb* antigens ESAT-6 and Ag85B, which is being developed as a pre- and post-exposure vaccine candidate for adolescents and young adults by Statens Serum Institut (SSI, Copenhagen). For clinical development in humans, H1 has been formulated in the two-component adjuvant IC31® (Intercell AG/Valneva) composed of the cationic polyaminoacid KLK, and the oligodeoxynucleotide ODN1a. In preclinical murine and guinea pig models, in comparison to BCG alone, H1:IC31 was safe and induced protective immune responses against *M.tb* challenge [12]. Subsequently, four clinical trials with H1:IC31 have been completed; three phase I trials [13,14] (unpublished data) and a phase II multicentre trial in HIV infected participants [15]. These studies demonstrated that a two-dose regimen with 50 μg of H1 protein in IC31 (500 nmol KLK and 20 nmol ODN1a) was safe in BCG vaccinated and unvaccinated adults, as well as persons with evidence of previous *M.tb* infection, known HIV seropositivity and individuals previously treated for TB. The vaccine also induced strong and persistent antigen-specific Th1 immune responses.

In this large phase II trial, we set out to determine the safety and tolerability of H1:IC31 and define the characteristics of induced immune responses in uninfected and *M.tb*-infected adolescents from a setting where TB is endemic. We explored effects of vaccine antigen dose, administration of one or two H1:IC31 vaccinations and determined the effects of pre and post *M.tb*-exposure vaccination on safety and immunogenicity of H1:IC31.

2. Methods

2.1. Trial design and participants

This was a phase II, single centre, randomized, observer-blinded (blinded to the subjects and those recording adverse events/drawing, processing blood samples and performing primary analyses), placebo-controlled clinical trial to evaluate safety and immunogenicity of H1:IC31 in healthy adolescents, who had received routine BCG at birth (Protocol available in supplementary material). We aimed to enrol 240 adolescents for randomization into one of four study groups. Group 1 received 2 vaccinations of 15 μg of H1, while Group 2 received 2 vaccinations of 50 μg of H1, 56 days apart. Groups 3 and 4 received only one vaccination of 15 μg, or 50 μg of H1, respectively and saline placebo on day 56. All H1 doses were formulated in a standard concentration of IC31 adjuvant (500 nmol KLK, 20 nmol ODN1a). At enrolment, participants were randomized into one of the study groups. Randomisation procedures took into account that the first half (120) of subjects enrolled should be Quantiferon Gold In-tube negative (QFT−) and the second half (120) QFT-positive (QFT+).

Prior to screening, participants and their guardians underwent an in-depth informed consent process. Only adolescents who provided written assent and whose legal guardians provided written consent, were screened for enrolment. The study was approved by University of Cape Town Faculty of Health Sciences Human Research Ethics Committee and South African Medicines Control Council (South African National Clinical Trials Register, DoH-27-0612-3947; Pan African Clinical Trial Registry, PACTR201403000464306) and conducted in accordance with Helsinki Declaration and Good Clinical Practices at the South African Tuberculosis Vaccine Initiative (SATVI) in the Breede Valley region, Western Cape, South Africa.

2.2. Inclusion and exclusion criteria

Participants were healthy adolescents aged 12–18 years. Adolescents were excluded if medical abnormalities were identified at screening through medical history and examination, chest X-ray, urine dipstick tests and safety blood tests (chemistry and haematology). Adolescents were excluded if found to be HIV-positive, or had evidence of previous TB disease, or pregnant, as were female participants who were sexually active and not willing to use contraception during the trial period.

2.3. Vaccination

H1:IC31 was administered on day 0 and/or day 56 by intramuscular injection into the deltoid area on alternate arms using syringe and needle (22–25 gauge 1–1.5 in.). Vaccine was stored in a temperature monitored freezer in the SATVI Pharmacy at <−15 °C and protected from light. Amongst study personnel, only pharmacists were not blinded.

Follow-up visits were conducted 1 and 14 days after each vaccination as well as on days 70, 112, 168 and 224 to examine the injection site, obtain a history of adverse events, perform physical examination, and review the participant diary card. Blood samples for safety assessments were taken at baseline (day 0) and all post-vaccination visits except Day 1 and Day 57. All adverse events (AE) and serious adverse events (SAEs) were recorded and reported. AEs were graded by an investigator for: type, causality, seriousness, severity and outcome using FDA's Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers (2005).

2.4. Immunogenicity assays

Venous blood was collected on days 0, 14, 56, 70, 112 and 224 for the trial's primary immunogenicity endpoint, the IFN-γ ELISpot on PBMCs and the secondary immunogenicity endpoint, a qualified whole blood intracellular cytokine staining (WBA-ICS) assay [16,17]. Detailed methods are available as supplementary data.

2.5. Data analysis

Data were analysed with Stata (v11 StataCorp), Prism (v 6.0f GraphPad Software Inc.) or R [18]. Flow cytometry analyses were

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