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Indicators of therapeutic effect in FIT-06, a Phase II trial of a DNA vaccine, GTU®-Multi-HIVB, in untreated HIV-1 infected subjects

Eftyhia Vardas[a,b,*], Ioana Stanescu[c], Mika Leinonen[d], Kim Ellefsen[e], Giuseppe Pantaleo[e], Minna Valtavaara[c], Mart Ustav[f], Kalevi Reijonen[c]

[a] Lancet Laboratories, Richmond, Johannesburg, South Africa
[b] Division of Medical Virology, University of Stellenbosch, Faculty of Health Sciences, South Africa
[c] FIT Biotech Ltd., Tampere, Finland
[d] 4 Pharma Ltd., Turku, Finland
[e] Centre Hospitalier Universitaire Vaudois, CH - 1011 Lausanne, Switzerland
[f] FIT Biotech Ltd., Tartu, Estonia

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ABSTRACT

Background: Combination highly active antiretroviral therapy (HAART) has significantly decreased HIV-1 related morbidity and mortality globally transforming HIV into a controllable condition. HAART has a number of limitations though, including limited access in resource constrained countries, which have driven the search for simpler, affordable HIV-1 treatment modalities. Therapeutic HIV-1 vaccines aim to provide immunological support to slow disease progression and decrease transmission. We evaluated the safety, immunogenicity and clinical effect of a novel recombinant plasmid DNA therapeutic HIV-1 vaccine, GTU®-multi-HIVB, containing 6 different genes derived from an HIV-1 subtype B isolate.

Methods: 63 untreated, healthy, HIV-1 infected, adults between 18 and 40 years were enrolled in a single-blinded, placebo-controlled Phase II trial in South Africa. Subjects were HIV-1 subtype C infected, had never received antiretrovirals, with CD4 ≥ 350 cells/mm^3 and pHIV-RNA ≥ 50 copies/mL at screening. Subjects were allocated to vaccine or placebo groups in a 2:1 ratio either administered intradermally (ID) (0.5 mg/dose) or intramuscularly (IM) (1 mg/dose) at 0, 4 and 12 weeks boosted at 76 and 80 weeks with 1 mg/dose (ID) and 2 mg/dose (IM), respectively. Safety was assessed by adverse event monitoring and immunogenicity by HIV-1-specific CD4+ and CD8+ T-cells using intracellular cytokine staining (ICS), pHIV-RNA and CD4 counts.

Results: Vaccine was safe and well tolerated with no vaccine related serious adverse events. Significant declines in log pHIV-RNA ($p = 0.012$) and increases in CD4+ T cell counts ($p = 0.066$) were observed in the vaccine group compared to placebo, more pronounced after IM administration and in some HLA haplotypes (B*5703) maintained for 17 months after the final immunisation.

Conclusions: The GTU®-multi-HIVB plasmid recombinant DNA therapeutic HIV-1 vaccine is safe, well tolerated and favourably affects pHIV-RNA and CD4 counts in untreated HIV-1infected individuals after IM administration in subjects with HLA B*57, B*8101 and B*5801haplotypes.

1. Introduction

Combination highly active antiretroviral therapy (HAART) for the treatment of HIV-1 infection has successfully controlled viral replication and extended life for infected individuals who have access to treatment [1,2]. However, HAART with current available drug combinations is limited in its success because; these drugs are unable to eradicate HIV-1 due to viral persistence in protected virus reservoirs like the brain and gut [3]; there is destruction of the immune cells necessary to mount an effective immune response to HIV-1 in spite of treatment; lifelong treatment with HAART drugs that potentially may cause short- and long-term side effects is necessary and patient adherence to multiple drugs with significant side effects is needed to prevent the development of drug resistant virus [4–6]. The cost and potential failings of HAART have provided the impetus for the development of therapeutic HIV-1 vaccines and other immune modulators that may be administered during chronic HIV-1 infection to decrease viral load thus decreasing the potential to transmit HIV-1 sexually or horizontally and decelerating progression to AIDS [5,7]. Therapeutic HIV-1 vaccines may be most relevant as an adjunct to antiretroviral treatment in

* Corresponding author at: Lancet Laboratories, PO Box 8475, Johannesburg, 2000, South Africa. Tel.: +27 11 242 7351; fax: +27 11 242 7355.
E-mail addresses: eftyxia.vardas@lancet.co.za, vardas@sun.ac.za (E. Vardas).

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resource constrained settings, where the HIV-1 prevalence is high and programmatic and drug costs limit access to HAART [8].

The goal of therapeutic HIV-1 vaccines is to induce recovery of HIV-1-specific immune responses [6,9,10]. Early therapeutic HIV-1 vaccines were evaluated in individuals that had not received HAART [11,12] and 'auto-immunisation', the concept that multiple re-exposures to an individual's own circulating HIV-1 antigens following structured treatment interruption (STI) could improve virus-specific immune responses and promote disease control [13,14], was of limited success [11,14,1]. Further the risk of viral rebound associated with STI's [13] led to therapeutic HIV-1 vaccine research focussing on complex interventions like dendritic cell vaccines which have had some initial success [15,16] but which would be difficult and costly to provide on a large scale to HIV-1 infected individuals. The design of therapeutic HIV-1 vaccine candidates in animals and humans has been predominantly naked DNA and viral vector-based [17–19]. But affordable therapeutic HIV-1 vaccines that may easily be administered in chronically infected individuals that do not have access to treatment or used in combination with modified HAART regimens to achieve viral suppression [12,20,21], primarily for areas where there is limited access to HAART [22] have not been sufficiently explored.

The aim of this study was to evaluate the safety, immunogenicity and clinical effect of a novel DNA vaccine GTU®-multi-HIV-1 B in healthy, chronically HIV-1-infected individual from South Africa that had never received HAART. The vaccine, a DNA plasmid vaccine, was designed to induce immune responses to HIV-1 regulatory genes *rev*, *nef* and *tat* and selected T-helper (Th) cell and CTL epitopes coded for by *pol* and *env* genes [19,20].

2. Materials and methods

This protocol was approved the ethics committee of the University of the Witwatersrand, Johannesburg and the national regulatory authority of South Africa.

2.1. Vaccine

A prototype GTU® vaccine containing only the *nef* gene was shown to be safe and well tolerated in Phase I in healthy, HAART treated HIV-1 infected individuals from Finland [23]. The prototype was expanded to include more HIV-1 structural and regulatory genes to elicit stronger, broader immune responses and provide cross-subtype reactivity. Two regulatory genes *rev* and *tat*, parts of the structural proteins coded by *gag* and a stretch of selected T helper (Th) and CTL epitopes coded for *pol* and *env* were added. All genes were derived from an HIV-1 subtype B virus (Han-2 isolate) [24]. E2, a transcriptional regulator from bovine papilloma virus (BPV) [25], was added to enhance transcription and expression of the cloned HIV-1 antigens, and facilitate prolonged antigen expression [26].

2.2. Subjects

Chronically HIV-1 infected subjects not on HAART treatment because of CD4 counts above 200 cells/mm^3 as per national guidelines [27] were recruited from an existing free Wellness Clinic in Soweto, Johannesburg. Subjects provided written informed consent and were included if they were healthy, with no intercurrent or chronic illness, between 18 and 40 years, no history of HAART, CD4 counts $\geq 350 \times 10^6$/L and plasma viral load (pHIV-RNA) $\geq$50 copies/mL at screening to exclude elite controllers, but not viraemic controllers with isolated episodes of plasma viral load (pHIV-RNA) >2000 copies/mL [28]. Further eligibility criteria were, no current or past AIDS defining illness, hepatitis B virus (HBV) or hepatitis C virus (HCV) infection and no previous participation in an HIV-1 vaccine or immune-modulatory trial, no active immunisation in the past month and no treatment with systemic corticosteroids in the past 6 months. Free barrier and hormonal contraceptives were available to participants during the study and participants were informed that the candidate vaccine risks in pregnancy were unknown.

2.3. Study design

This was a single centre, randomised, placebo-controlled, single-blinded, Phase II study in untreated HIV-1 subtype C chronically infected individuals. Two different administration routes with the Biojector, a device to ensure standardisation and needle-free administration, were assessed. Placebo was phosphate buffered saline (PBS). Thirty-one subjects were randomised to receive the study intervention intradermally (ID) (21 vaccine and 10 placebo) and 32 subjects intramuscularly (IM) (21 vaccine and 11 placebo). A computer-generated blocked randomisation was used. Five immunisations were administered to each subject; a 3 dose priming schedule at 0, 4 and 12 weeks (500 μg/dose ID, or 1000 μg/dose IM), followed by two boosts at 76 and 80 weeks (1000 μg/dose ID and 2000 μg/dose IM) were given (Fig. 1).

Subjects were monitored for 16 visits over 27 months (108 weeks). As per protocol, if CD4 cells dropped between 300–250 $\times 10^6$/L they were referred for HAART. Safety was evaluated at every vaccination visit by clinical criteria, laboratory safety variables and the occurrence local or generalised adverse events (AE's).

2.4. Laboratory assays

Four colour flow cytometer CD4, CD8, T cell counts (BD MultiTEST), pHIV-RNA (Roche, COBAS Amplicor Ultrasensitive), full blood count, liver function tests and creatinine were performed at a local accredited clinical laboratory.

2.4.1. Immunogenicity evaluation

Immunogenicity of the GTU®-multi-HIVB vaccine was assessed by intracellular cytokine staining (ICS) analyses of IL-2, TNF-α and IFN-γ expression by HIV-1-specific CD4+ and CD8+ T-cells at 6 time points, (13, 16, 40, 64, 76 and 84 weeks). Samples were processed within 24 h after shipping to the National Institute of Communicable Diseases (NICD) Johannesburg, South Africa. Peripheral blood monocyte cells (PBMC's) were isolated by Ficoll centrifugation and stored in liquid nitrogen (≤−140 °C).

Cryo-preserved peripheral blood mononuclear cells (PBMC's) were shipped to the Vaccinology and Immunotherapy Center (VIC), Lausanne, Switzerland on dry-ice and stored in liquid nitrogen (≤−140 °C) until analysis. All samples were then analysed together, cryo-preserved PBMC's were thawed and rested overnight. PBMC viability before and after resting was assessed by an in-house trypan blue exclusion method. Viable PBMC's (0.5×10^6–1.5×10^6) were stimulated for 6 h in 1 mL RPMI plus 10% fetal bovine serum in the presence of Golgi plug (1 μL/mL; BD), αCD28 (0.5 μg/mL; BD), and 1 μg of peptide pool/mL. For each sample, peptide pools were tested separately for Gag-B, Gag-C, CTL-B and CTL-C if sufficient viable cells were obtained. Staphylococcal enterotoxin B (SEB) stimulation (200 ng/mL) and media alone were used as positive and negative controls, respectively. After stimulation staining for dead cells using the AQUA LIVE/DEAD stain kit (Invitrogen) was done. Staining for CCR7 was also done then cells were permeabilised (Cytofix/Cytoperm, BD) and stained with CD14, CD19, CD3, CD4, CD8, IFN-γ, TNF-α, CD154 and IL-2 monoclonal antibodies. Cells were fixed, and analysed on a 4 laser flow cytometer (LSRII SORP) with FlowJo 8.7.1. software. The number of