A FIRST-IN-HUMAN PHASE I/II TRIAL DEMONSTRATES THE SAFETY & THE IMMUNOGENICITY OF A LENTIVIRAL-BASED THERAPEUTIC HIV VACCINE ELICITING POTENT POLYFUNCTIONAL MULTISPECIFIC CD8 & CD4 T-CELL RESPONSES IN HIV-INFECTED INDIVIDUALS

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ABSTRACT

BACKGROUND: One approach to reach the functional cure in HIV-infected individuals is the development of **T-cell immune-based strategies** able to contain viral replication while preserving CD4+ cells. We assessed the **safety and efficacy** of a therapeutic anti-HIV-1 prime-boost vaccine regimen based on intramuscular injection of **two integrative lentiviral vectors** (ClinicalTrials.govIdentifier: NCT02054286).

METHODS: The randomized, placebo-controlled trial enrolled **38 HIV-infected individuals** on suppressive ART and aimed at comparing the safety, tolerability and immunogenicity of the therapeutic vaccine candidate at **3 incremental doses** (5.10^6, 5.10^7 or 5.10^8 TU) versus placebo.The vaccination regimen consisted of two intramuscular injections **8 weeks apart with non-replicative and self-inactivating lentiviral vectors** encoding for immunogenic regions of the HIV GAG, POL and NEF proteins under the regulation of the **ß2-microglobulin human promoter**. Vaccine-induced HIV-specific T-cell in peripheral blood were characterized by **intracellular cytokine** staining in all participants, placebo included, before and after ART interruption and **up to 24 weeks after the first injection**.

RESULTS: With the **lack of any serious adverse events** in all 38 participants and **no safety concerns related to the treatment**, the clinical data **confirmed safety and tolerance** of the lentiviral-based therapeutic vaccine. Analysis of the immunological data demonstrated the ability of the vaccine to **elicit multi-specific and poly-functional cellular immune responses in vaccinated subjects**. The vaccine candidate was highly immunogenic at all doses when compared to the placebo group: i) 93% of the vaccinated subjects showed vaccine **specific CD4+ and CD8+ T-cell responses** compared to 66,6% of the placebo group; ii) a high frequency, from 0.097 to 0.874%, of functional T-cells able to produce at least 2 or 3 cytokines among IFN-γ, TNF-α and IL-2 was evidenced; iii) a dose effect was observed when comparing the 3 groups, with greater magnitude with the highest dose; iv) sustainable responses were characterized up to 24 weeks.

CONCLUSIONS: **This first-in-human study demonstrates the safety, tolerability and immunogenicity of a lentiviral-based therapeutic vaccine regimen.** We are currently evaluating the impact of ART interruption of vaccination on CD4 T-cell levels, plasma viral load and viral reservoirs of the induced immune response to optimize the design of the planned Phase II.

THERAVECTYS TECHNOLOGY

Integrative recombinant lentiviral vectors developed by THERAVECTYS are derived from the **HIV-1 NL4-3 strain**. They are non-replicative, non-pathogenic and self-inactivating *(Fig.1)*. They are able to transduce both dividing and non-dividing cells thanks to dubbed DNA Flap, a 99 nucleotide long sequence identified in 1999 at Pasteur Institute, which is formed during the natural reverse transcription of HIV. This sequence is necessary for the virus' pre-integration complex to cross the nucleus membrane of the non-dividing cells it's infecting.

The ability of lentiviral vectors to transduce dendritic cells in a stable manner enables the presentation of antigens in an endogenous way to T cells, thereby allowing the first vaccine applications to be developed. The antigens encoded by THERAVECTYS lentiviral vectors are under the regulation of THERAVECTYS' patented human promoter (THV-PROM) that is overexpressed in APC.

Furthermore, THERAVECTYS has developed an efficient prime-boost regimen that enables iterative injections of the same product. Thus, vaccines developed by THERAVECTYS allow the generation of a long lasting immune response that is both strong and specific.

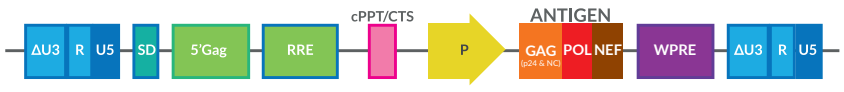


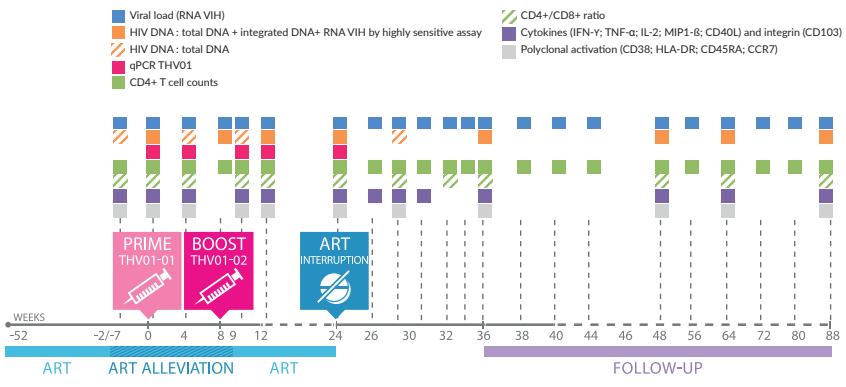
Figure 1: Schematic representation of Theravectys lentiviral vector. Sequences from 3' LTR were deleted to make this vector noninfectious. cPPT/CTS sequences were added to improve the transduction capacity.

THERAVECTYS has designed an innovative manufacturing process combining high production yields, impurity profiles compatible with direct injection into humans or genetic modification of immune cells, and high immunogenicity. In parallel, THERAVECTYS has developed specific assays to control the quality of lentiviral vectors and lentiviral vector-modified T-cells. Moreover THERAVECTYS has set up a GMP bioproduction facility dedicated to the production of lentiviral vectors and lentiviral vector-modified immune cells. THERAVECTYS manufacturing facility will integrate both production and QC activity.

STUDY DESIGN & OBJECTIVES

This randomized, double-blind, placebo-controlled Phase I/IIa trial was conducted to compare the **safety**, **tolerability** and **immunogenicity** of **therapeutic vaccination** at 3 escalating doses (5.10^6 TU, 5.10^7 TU or 5.10^8 TU) versus placebo. A total of **38 patients HIV-1 clade B infected** under highly active antiretroviral therapy (ART) were enrolled in 12 sites in France and Belgium. The vaccine encodes HIV-1 clade B sequences derived from **Gag** (p24 and NC) and epitopic fragments of **Pol** and **Nef**. The prime-boost regimen consists in **2 intramuscular injections** of **2 lentiviral vectors** encoding for the same transgene with **2 different envelops** *(Fig. 2)*.

Figure 2: Outline describing vaccinations and follow-up of the study. The vaccine was administered at week 0 and 8 weeks later. Sampling for plasma HIV viral load, blood HIV DNA, CD4+ T cell counts, polyclonal activation and vaccine-induced HIV-specific T-cell immunity were evaluated in all participants, placebo included, before and after ART interruption and up to 24 weeks after the first injection.



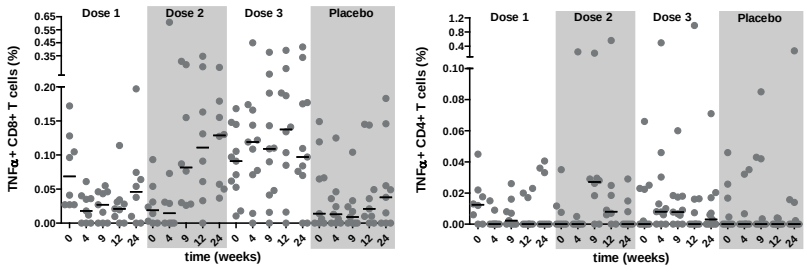
RESULTS

Vaccine safety:

Primary endpoint related to safety was assessed before baseline from **week -7** or **week -2**, after baseline to ART interruption at **week 24** and after **week 24** to **week 36** or early termination. **No serious adverse events has been reported**. The distribution of treatment emergent adverse event incidence was globally similar in the four treatment groups, except that injection site pain occured in 8/11 participants at the highest dose. The vaccine treatment was **well tolerated** both at the local site of injection and at the systemic level for every dose.

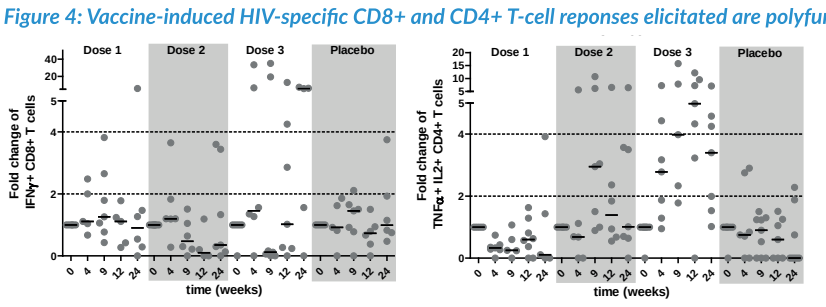
Immunogenicity:

Figure 3: Candidate vaccine elicits strong CD8+ and CD4+ T-cell immune responses in 93% of all vaccinated patients.



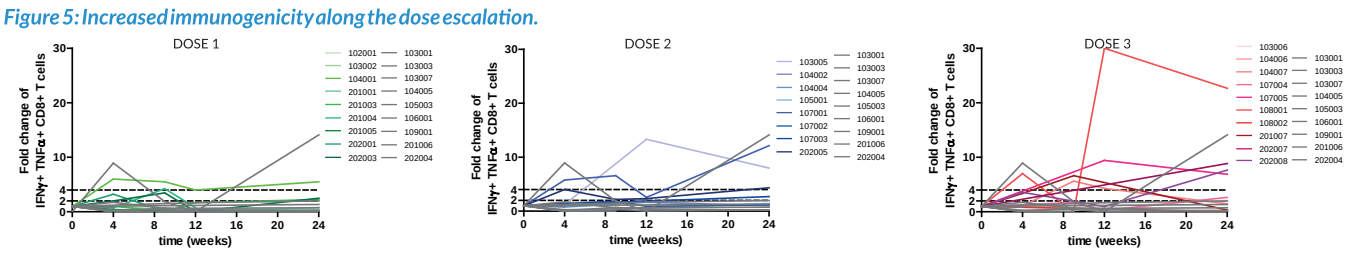
IFNγ, TNFα or IL-2 secretion capacity of HIV-specific CD8+ and CD4+ T cell was assessed by intracellular staining (ICS) after ex-vivo stimulation of whole blood samples with pool of peptides for all patients and placebo at each timepoint of the study. Frequency of Gag specific TNFα+ CD3+ CD8+ cells A and frequency of Pol specific TNFα+ CD3+ CD4+ cells B from week 0 to week 24 are shown. Each dot represent an individual patient and horizontal black lines indicate medians at indicated timepoint. Frequency for each antigens specific TNFα+ CD3+ CD8+ cell responses for an individual patient from dose 2 (5.10^7 TU) C and antigens specific TNFα+ CD3+ CD4+ cell responses for an individual patient from dose 3 (5.10^8 TU) D are presented.

Figure 4: Vaccine-induced HIV-specific CD8+ and CD4+ T-cell reponses elicitated are polyfunctional and multi-antigenic.



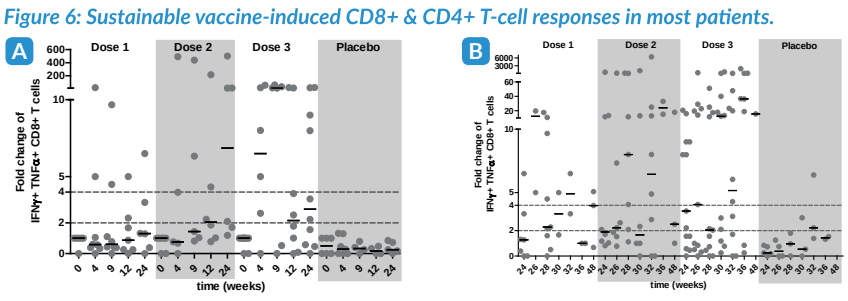
CD8+ and CD4+ T cell polyfunctionality of 38 patients samples was monitored for each timepoint and to each encoded vaccine antigens by ICS for IFNγ, TNFα or IL-2 cytokines. Fold change from baseline at week 0 of frequency of Gag-specific IFNγ+ CD3+ CD8+ cells A and Pol-specific TNFα+/IL-2+ CD3+ CD4+ cells B measured from week 0 to week 24 are shown. Dashed lines denote 2 or 4 fold thresholds from baseline. Representative fold change from baseline at week 0 of frequency of antigens-specific IFNγ+/TNFα+ CD3+ CD8+ cells of a dose 2 (5.10^7 TU) vaccinated patient C and fold change from baseline at week 0 of frequency of antigens-specific IFNγ+/TNFα+ CD3+ CD4+ cells of a dose 3 (5.10^8 TU) vaccinated patient D are shown.

Figure 5: Increased immunogenicity along the dose escalation.



Fold change from baseline at week 0 to week 24 of frequency of Pol-specific IFNγ+/TNFα+ CD3+ CD8+ cells for all subjects at dose 1 (5.10^6 TU) A dose 2 (5.10^7 TU), B and dose 3 (5.10^8 TU) C and placebo. Colored lines represent individual vaccinated subjects while gray lines represent each individual placebo. Dashed lines indicate 2 or 4 fold thresholds from baseline.

Figure 6: Sustainable vaccine-induced CD8+ & CD4+ T-cell responses in most patients.



IFNγ, TNFα or IL-2 secretion capacity of HIV-specific CD8+ and CD4+ T cell was assessed by ICS after ex-vivo stimulation of whole blood samples with pool of peptides for all patients and placebo at each timepoint of the study from week 0 to week 24 A, or post-week 24 after ART interruption B. Nef specific IFNγ+/TNFα+ CD3+ CD8+ cell responses are presented. Each gray circle represent an individual patient. Horizontal black lines indicate medians at each timepoint. Dashed lines shows 2 or 4 fold thresholds from baseline.

CONCLUSION

These clinical data for a **first-in-human lentiviral-based therapeutic vaccine support the safety of lentivector injections in a prime-boost regimen**.
This novel candidate vaccine demonstrated elicitation of:

- ***intense*** *specific CD4+ and CD8+ T-cell responses,*
- ***polyfunctional*** *specific CD4+ and CD8+ T-cell responses, with coproduction of IFNγ and TNFα,*
- *broad CD4+ and CD8+ T-cell responses with* ***multiple vaccine antigens targeted****,*
- ***persistant*** *specific CD4+ and CD8+ T-cell responses beyond 24 weeks after the first injection, and*
- ***dose-dependent*** *immunogenicity with highest frequency of immune responders at the highest dose.*

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