Abstract 3630 (Late Breaker)

VAC-3S, a safe Immunotherapeutic HIV Vaccine decreased total HIV DNA and increased CD4/CD8 ratio: Phase I Final Results.

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INTRODUCTION

We have developed an innovative immunotherapy based on a highly specific and conserved motif, called 3S, located in the gp41 HIV-1 protein. This highly pathogenic motif induces expression of NKp44L, the cellular ligand of an activating NK receptor (NKp44), rendering uninfected CD4⁺ T cells sensitive to NK lysis[1,5,7,13] (**Figure 1**). NKp44L expression is strongly correlated with both the decline of CD4 cell count[2,6,8,9,11,15,18], and additional effects linked to the pathogenecity (ie. T-cell activation, inflammation, and apoptosis of CD4⁺ T cells). These data were observed *in vitro* after 3S stimulation[1,5,7], *ex-vivo* in HIV-infected patients[1], and *in vivo* in SHIV-infected macaques[4]. A protection against CD4 depletion and chronic immune activation was demonstrated in SHIV-infected macaques immunized with a 3S vaccine[3,10]. Non clinical data[3,10,14,17], as well as the safety and immunogenicity results[19-25] of this study have been previously presented. We present here the final efficacy results of the phase I/IIa clinical trial IVVAC-3S/P1.

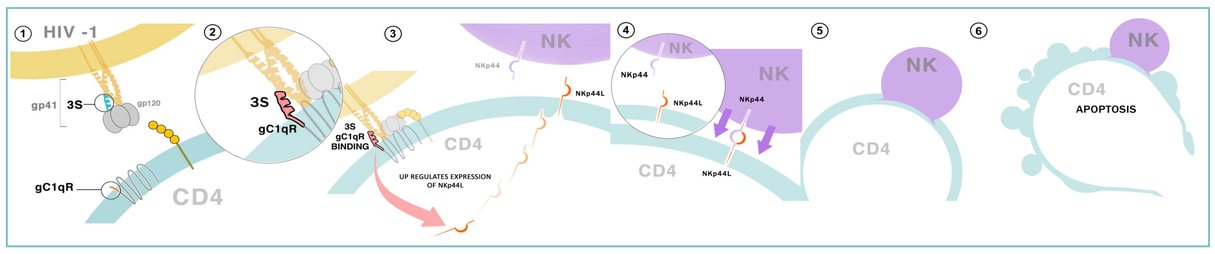

Figure 1 : Depletion of uninfected CD4⁺ T cell by 3S

Steps ① & ②: The gp41 3S motif of HIV-1 binds to its specific receptor (gC1qR) on CD4⁺ T cells.
Step ③: This interaction induces NKp44L expression at the surface of non-infected CD4⁺ T cells.
Steps ④ to ⑥: NKp44L/NKp44 interaction provokes NK-mediated cytotoxicity.

METHODS

IVVAC-3S/P1 was a prospective, randomized, placebo-controlled, double-blind, dose-escalation study, to assess safety and immunogenicity of 3 VAC-3S intramuscular administrations (weeks 0, 4, 8) at 0.1, 1, 10 and 20 µg and 1 booster (week 32) in 1 and 10 µg arms, in patients receiving ART with CD4 > 200 cells/mm³ and virologically controlled. Analysis included safety, anti-3S antibodies (ELISA), CD4/CD8 ratio, T lymphocyte activation/differentiation, total HIV DNA and inflammation biomarkers.

Responders were defined as patients with total anti-3S antibodies above or equal to 30 arbitrary units (AU) at week 12 from the active arms. Non-responders were defined as patients with total anti-3S antibodies below 30 arbitrary units (AU) at week 12 from the active arms.

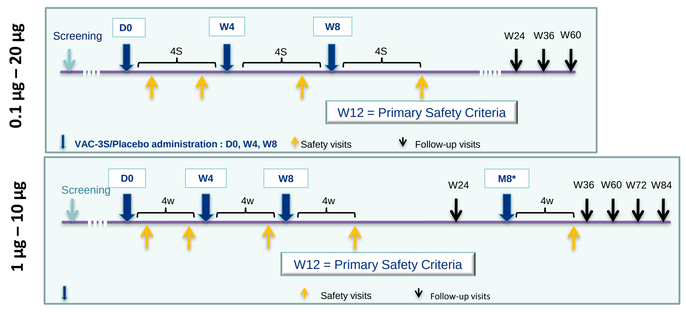

Figure 2 : Study Plan

Upper panel represents the study plan for patients of the 0.1 µg and 20 µg arms with 3 VAC-3S/Placebo administration. Lower panel represents the study plan for patients of the 1 µg and 10 µg arms with 4 VAC-3S/Placebo administration.

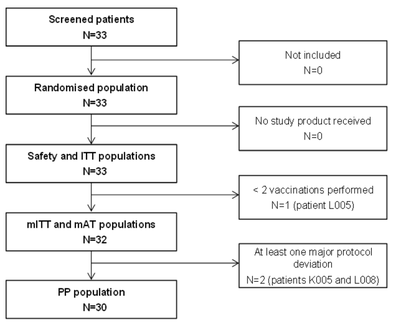

Figure 3 : Patient Disposition. Presented efficacy analyses are performed in mAT (modified as treated) population.

RESULTS

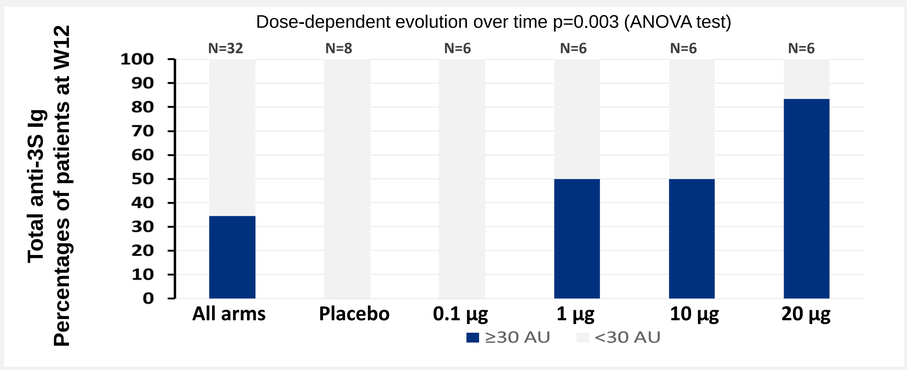

Figure 5 : VAC-3S is immunogenic. Total Anti-3S Ig were measured by ELISA. The percentage of patients with Total anti-3S Ig titers above 30 Arbitrary Units is represented (blue bar) in all patients (N=32), placebo arm (N=8), 0.1 µg arm (N=8), 1 µg arm (N=6), 1 µg arm (N=6), 10 µg arm (N=6) and 20 µg arm (N=6). Temporary threshold of 30 A.U. is arbitrary and is above the limit of quantification of the ELISA technique.

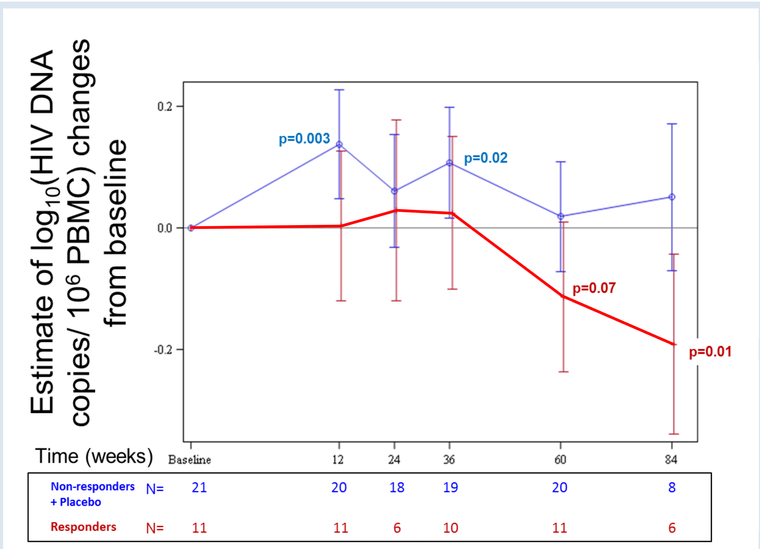

Figure 7 : VAC-3S decreases HIV reservoir. Total HIV-1 DNA copies were measured by PCR from frozen PBMCs. Estimate of changes from baseline of log_{10}(HIV-1 DNA copies/10^6 PBMC) are plotted for each time points for the responder (red line) and the non-responders + placebo groups (blue line). Error bars represent 95% interval confidences. See figure 7 for individual changes from baseline at week 60 and week 84. Results are confirmed when expressed in HIV DNA copies /mL. (ANOVA test)

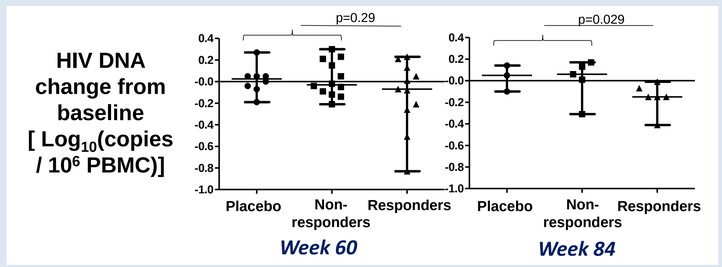

Figure 8 : Changes from baseline of HIV DNA/10^6 PBMC in the 3 groups. Individual data are plotted, the bar represents the median. (Mann-Whitney test)

Randomised Population

Patient Disposition Median [Range]	Dose 1 0.1 µg (N=6)	Dose 2 1 µg (N=6)	Dose 3 10 µg (N=6)	Dose 4 20 µg (N=6)	Placebo 0 µg (N=9)
Gender H/F	6 / 0	4 / 2	6 / 0	5 / 1	8 / 1
Age (years)	**40** [32-53]	**48** [40-54]	**46** [35-49]	**41** [30-53]	**49** ± 6
Weight (kg)	**75** [57-88]	**75** [55-98]	**72** [64-80]	**74** [44-83]	**71** [67-86]
Body Mass Index	**25** [19-30]	**25** [19-36]	**24** [22-25]	**23** [20-26]	**24** [21-26]
Patients who received three administrations	6/6	6/6	6/6	6/6	8/9
Nadir CD4	**335** [235-418]	**275** [127-402]	**483** [129-739]	**331** [150-437]	**337** [150-536]

Source: Table 14.1.02.1 Section 14.1

mAT Population

BASELINE = Mean (Screening - D0) Median [Range]	Dose 1 0.1 µg (N=6)	Dose 2 1 µg (N=6)	Dose 3 10 µg (N=6)	Dose 4 20 µg (N=6)	Placebo 0 µg (N=8)
CD4+ T cell count (cells/mm3)	**708.0** [609 ; 1134]	**653.0** [362 ; 928]	**697.3** [590 ; 983]	**515.8** [444 ; 825]	**654.3** [432 ; 821]
CD8+ T cell count (cells/mm3)	**746.0** [433 ; 1142]	**884.3** [392 ; 1084]	**866.8** [519 ; 1493]	**488.8** [298 ; 1059]	**601.8** [374 ; 1033]
CD4+ T cell percentage	**38.5** [30 ; 42]	**34.8** [32 ; 45]	**34.5** [24 ; 48]	**37.5** [33 ; 44]	**38.5** [32 ; 44]
CD8+ T cell percentage	**38.8** [30 ; 44]	**46.3** [29 ; 53]	**42.8** [32 ; 55]	**35.8** [27 ; 50]	**35.5** [29 ; 46]
CD4+/CD8+ ratio	**1.003** [0.68 ; 1.43]	**0.705** [0.64 ; 1.31]	**0.810** [0.44 ; 1.55]	**1.053** [0.78 ; 1.66]	**1.058** [0.78 ; 1.50]

Source: Tables 14.3.03.55, 61, 67, 73, 79 Section 14.3

Tables 1 & 2 : Demography at baseline

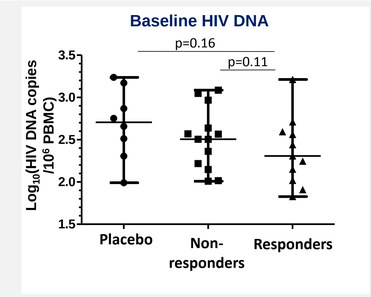

Figure 4 : Baseline of HIV DNA/10^6 PBMC in the 3 groups. Individual data are plotted, the bar represents the median. (Mann-Whitney test)

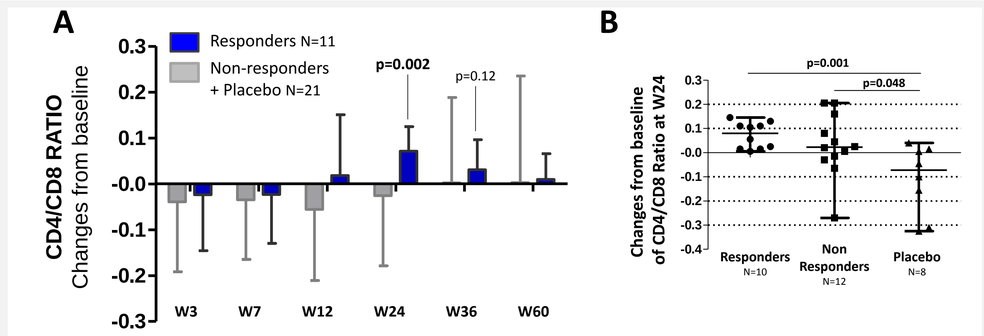

Figure 6 : VAC-3S increases CD4/CD8 Ratio. Percentages of $CD3^+CD4^+$ and $CD3^+CD8^+$ T cells were measured by cytofluorometry.

A. Mean changes from baseline of CD4/CD8 ratio and SD (error bars) are reported for each visit for the responder (blue bar) and the non-responders + placebo (grey bar) groups . (Wilcoxon paired test)
B. Individual changes from baseline are plotted at week 24. The bar represents the median. (One-tailed Mann-Whitney test)

A trend was found between change of CD4/CD8 ratio at W24 and anti-3S titers at W12 (r=0.29, p=0.11). **Globally, a dose-dependent decrease in CD8 % has been found (p<0.001, ANOVA test).** At W24, we observed a decrease on CD8 % (p=0.027, Wilcoxon paired test) and a significant increase in CD4 % (p=0.031, Wilcoxon paired test) in the responder group. CD4/CD8 ratio, CD8 % decreases and CD4 % decrease are also observed in 20 µg arm (p=0.03; p=0.03 and p=0.13 respectively; one-tailed Wilcoxon test). Increases in CD4/CD8 ratio and CD4 % as well as decrease in CD8 % were expected effects. **A negative correlation was found between change from baseline of $CD4^+CD95^+$ % and anti-3S titers at W24 (r=-0,59, p=0.002, Pearson test) suggesting protective effects of anti-3S antibodies of $CD4^+$ T cells against bystander apoptosis, in agreement with the mechanism of figure 1.** Further analysis are ongoing.

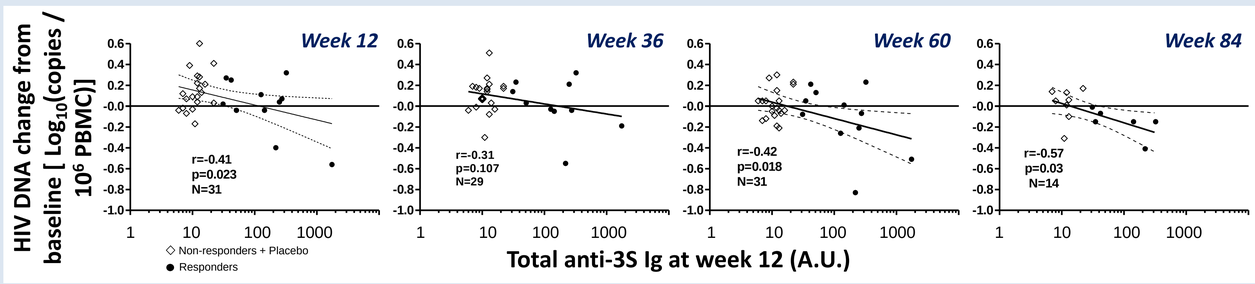

Figure 9 : Decrease in HIV reservoir correlates with anti-3S titers. Changes from baseline of log_{10}(HIV-1 DNA copies/10^6 PBMC) at weeks 12, 36, 60, and 84 were plotted against total anti-3S Ig titers at week 12, time of the peak of the antibody response, for all available patients. White diamonds : non-responders + placebo; filled circles: responders. Error bars represent 95 % interval confidences. Results are confirmed when expressed in HIV DNA copies / mL. (Pearson test)

Serum Inflammatory Markers concentrations were measured from frozen samples. **No significant changes could be detected.**

Ultra Sensitive Viral Load were performed by real-time RT-PCR with a limit of quantification of 2 HIV RNA copies/mL. Patients were undetectable using routine techniques. About 10 % of the tested time points were positive between 2 and 20 HIV RNA copies/mL. The too low level of residual replication did not permit to detect a decrease in the viral replication that could have been induced by the decrease observed in the viral reservoir. Nevertheless, **VAC-3S administration did not result in an increase in the residual replication.** (Data not shown)

Cellular Responses were tested from frozen PBMCs at D0, week 12 and week 60 by Intracellular Staining to IFNg, IL2, 4-1BB and TNFa after stimulation by 3S peptide, GAG, NEF and a pool of HIV peptides. No cellular responses to 3S were induced. **No significant changes could be detected.** (Data not shown)

CONCLUSION & PERSPECTIVES

VAC-3S is a safe and immunogenic HIV immunotherapy at higher tested doses. The induction of anti-3S antibodies was associated with increased CD4/CD8 ratio and decreased total HIV blood reservoir. *Post-hoc* analyses are being conducted in order to better characterize anti-3S antibody properties as well as virological and immunological effects. Our working hypothesis on the total HIV DNA decrease is a direct effect of anti-3S antibodies on latently infected cells and/or an effect mediated by the immune normalization.

A phase IIa on 90 patients is currently running to confirm safety, immunogenicity and biological effects in patients with CD4 counts between 200 and 500 cells/mm³(see poster MOPEA038).

A combination phase IIb trial with VAC-3S is planned to be launched in the arena of functional cure.

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