Experimental Evidence and Potential Immunotherapeutic Applications of Vaccine-Induced Antibodies Against 3S, a Highly Conserved Motif of gp41, in HIV-1-infected Patients Treated with Antiretroviral Therapy.

May 21>23, 2013 Institut Pasteur, Paris, France — 30 years of HIV science — Imagine the future

Poster No. 145

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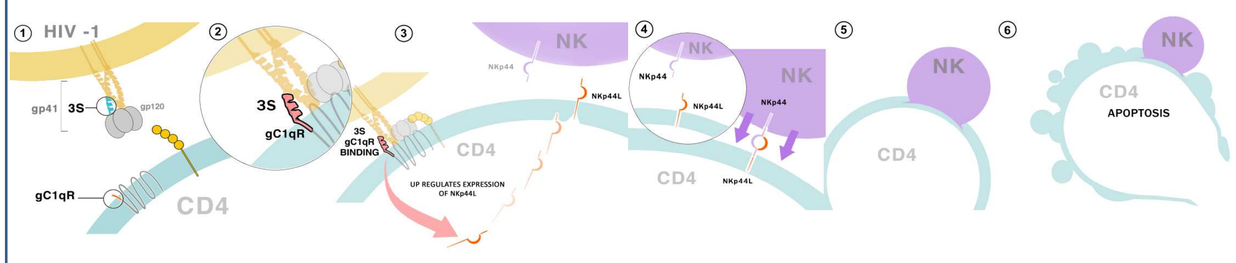
Background / Hypothesis

We hypothesize that 3S, a highly conserved motif of HIV-1 gp41, is a key target for an immunotherapeutic and immunoprotective vaccine (1-6). This peptide binds to gC1qR, leading to expression on CD4 of NKp44L, the natural ligand of NKp44 on activated NK cells, thus provoking CD4 apoptosis/depletion. Anti-3S antibodies prevent NKp44L expression and ensuing cytotoxic events (1).

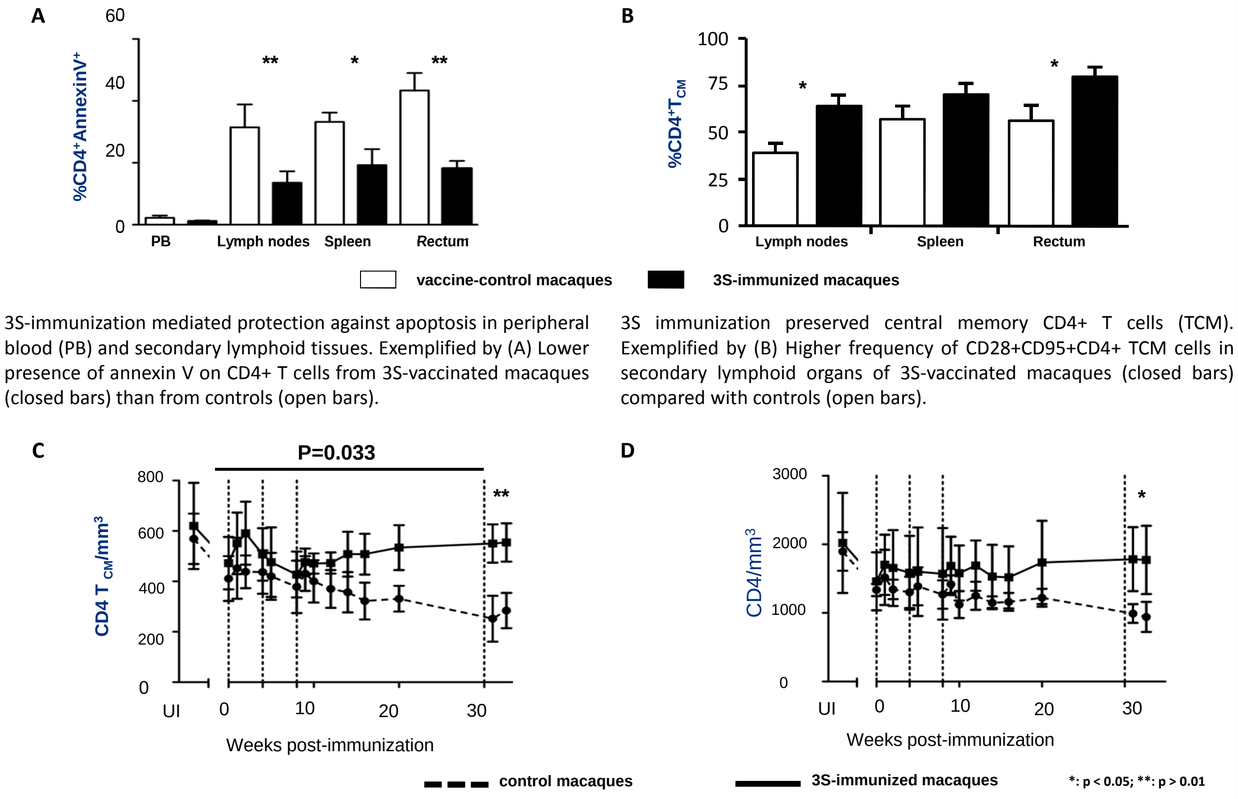
Figure 1: Steps in the Mechanism of Action of 3S During HIV-1 Infection and Potential Role for VAC-3S Vaccination



[Steps 1 & 2] The gp41 3S motif of HIV-1 binds to its specific receptor (gC1qR) on CD4⁺ T cells (CD4)
[Step 3] This interaction induces a molecular pathway that leads to NKp44L expression at the surface of CD4⁺ T cells
[Steps 4 to 6] NKp44L is the natural ligand of NKp44, which is expressed on activated Natural Killer cells (NK). NKp44L/NKp44 interaction provokes NK-mediated cytotoxicity via apoptosis and CD4⁺ T cell depletion. The apoptosis of CD4⁺ T cells may indirectly induce inflammation and chronic immune activation (1-3,5).

Proof-of-concept in an non-human primate model for AIDS was carried out with nine cynomolgus macaques that were chronically infected with the $SHIV_{163P3}$. Four immunized with the 3S/gp41 peptide containing vaccine and five with the control vaccine. CD4+ T cell subsets, proliferation, cell activation and apoptosis were analyzed in the peripheral blood, the lymph nodes, spleen and rectum by flow cytometry. Anti-3S antibodies were shown to prevent NKp44L expression on CD4+ T cells *in vivo* preserving the peripheral CD4+ central memory T cells in 3S/gp41-vaccinated animals. Anti-3S antibodies also limited the NK cytotoxic activity against autologous CD4+ T cells, CD4 activation, proliferation and apoptosis in secondary lymphoid tissues (8). VAC-3S dose ranging, GLP toxicity and local tolerance assessments were performed in rats/mice (9).

Figure 2: Selected Results Illustrating VAC-3S Proof-of-Concept in a Non-Human Primate Model for AIDS



3S-immunization mediated protection against apoptosis in peripheral blood (PB) and secondary lymphoid tissues. Exemplified by (A) Lower presence of annexin V on CD4+ T cells from 3S-vaccinated macaques (closed bars) than from controls (open bars).

3S immunization preserved central memory CD4+ T cells (TCM). Exemplified by (B) Higher frequency of CD28+CD95+CD4+ TCM cells in secondary lymphoid organs of 3S-vaccinated macaques (closed bars) compared with controls (open bars).

3S immunization inhibited depletion and activation of CD4+ T cells. Exemplified by (C) higher absolute central memory CD4+ T cell counts from peripheral blood of 3S-vaccinated macaques (solid lines or closed bars) compared with controls (dotted lines or open bars).

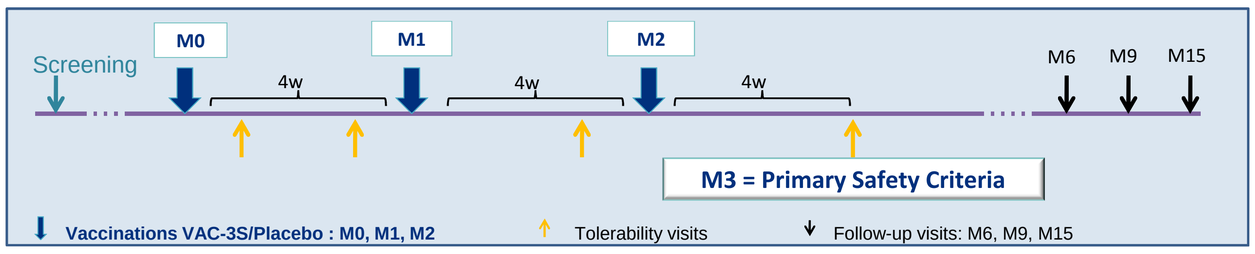
3S immunization preserved CD4+ T cell counts. Exemplified by (D) Higher absolute cell counts (A) in the peripheral blood of 3S vaccinated macaques (solid lines) and controls (dotted lines)

Clinical observational data: anti-3S antibodies in 5 cohort studies (Total N=923 patients) correlated with a lack of CD4 decrease and/or HIV disease progression (4,6, and InnaVirVax / Inserm data on file).
We have thus eveloped a novel immunotherapeutic vaccine (VAC-3S), comprised of 3S and commercially used carrier protein and adjuvant (7,10).

Methods

The First-Time-In-Human clinical trial is a prospective, randomized, placebo-controlled, double-blind dose-escalation study to assess safety, immunogenicity of 0.1, 1.0, 10 µg of VAC-3S with 3 IM immunizations, Week (W)0, W4, W8. Anti-3S antibodies were assessed by ELISA. Secondary endpoints include NKp44L expression, lymphocyte activation/differentiation.

Figure 3: Summary of VAC-3S Phase I/IIa Schedule



- Primary objective : safety & tolerability
- Secondary objectives : immunogenicity, plasma anti-3S Ab titers, NKp44L expression on $CD4^+$ T cells, $CD4^+$, $CD8^+$ T cell count and percentages, CD4/CD8 ratio, expression of markers of lymphocyte activation (CD25, CD38, HLA-DR) on $CD45^+CD3^+CD4^+$, $CD45^+CD3^+CD8^+$ and $CD45^+CD3^-CD8^+$ cells, expression of markers of lymphocyte differentiation (CCR7, CD45RA) on $CD45^+CD3^+CD4^+$ and $CD45^+CD3^+CD8^+$ cells.
- Inclusion and non-inclusion criteria included but were not limited to the following : Patients on antiretroviral therapy (ART) ≥ 1 year, CD4 count at entry ≥ 200 c/mm³, Nadir CD4 count ≥ 100 c/mm³, no immunotherapy within the past 12 months, no vaccination within the past quarter

Results

Twenty-five virologically controlled HIV-1 pts (23 men) receiving ART with CD4 counts >200 cells/mm³ were randomized. Median (min-max) age was 47 years (32-54), CD4 710 cells/mm³ (311-1187), CD4 nadir 336 cells/mm³ (127-739), ART duration median 3.0 yrs (1.1-7.1), none had detectable HIV RNA at inclusion.

Table 1: Demographic Characteristics in VAC-3S Study Groups, Safety Population (patients who received at least one injection).

Characteristics mean values ±SD*	Group Dose 1 0.1 µg ● N=6	Group Dose 2 1 µg ● N=6	Group Dose 3 10 µg ● N=6	Placebo Group 0 µg ● N=7
Gender M/F	6/0	4/2	6/0	7/0**
Age, yrs	41 ± 8	48 ± 5	44 ±5	49 ± 7
Weight. kg	74 ± 12	77 ± 15	71 ± 7	76 ± 7
BMI	25 ± 5	26 ± 6	24 ± 1	24 ± 2
Enrolled / Completed Vaccination Schedule	6/6	6/6	6/6	6/6
CD4 count nadir at baseline, cells/mm³	335 ± 60	283 ± 105	459 ± 229	332 ± 131

*No decimals included, values rounded up or down ** 1 patient was replaced

Results [continued]

Figure 4: Immunogenicity Results of VAC-3S. [modified as treated (mAT) population]

(patients who received at least two injections, the treatment received will be used in case of allocation of the wrong treatment)

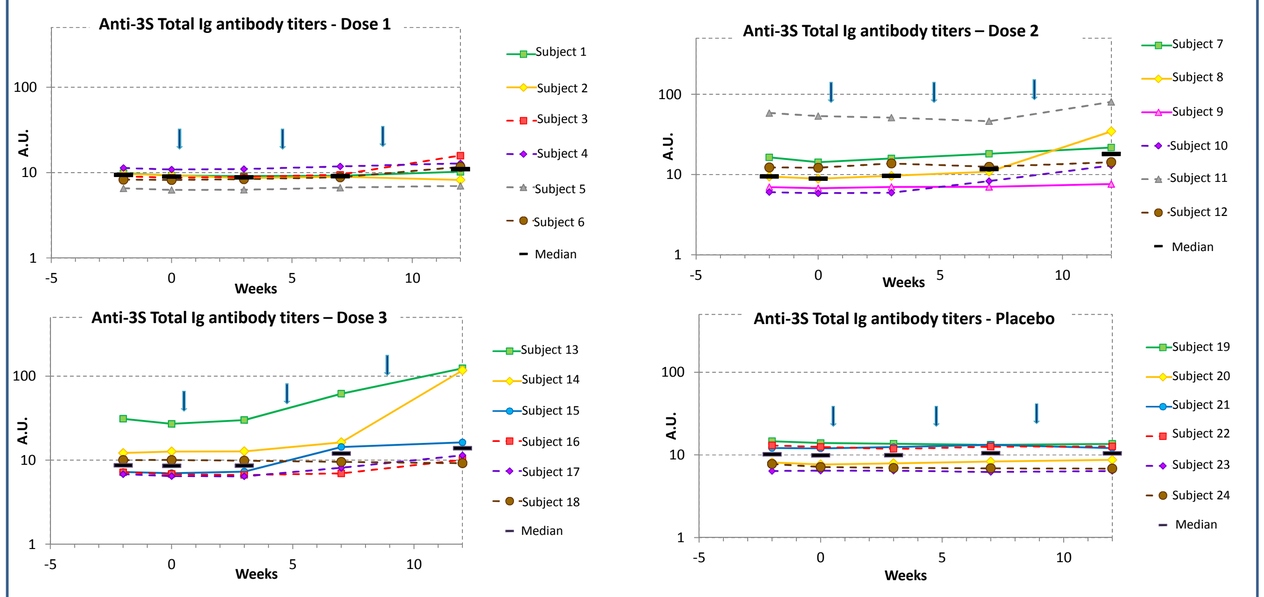


Figure 5: Total Plasma anti-3S Ab Titres (*Arbitrary Units, A.U.*) [Mean per dose over time – modified as treated (mAT) population]

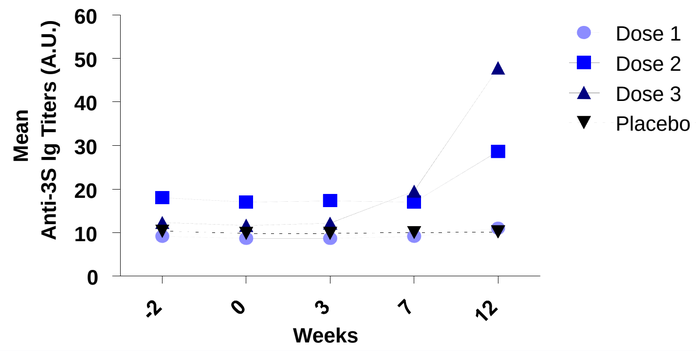


Table 2: Immunological Outcome with VAC-3S Vaccination [mAT population]

Unit: cells /mm³

		Group Dose 1 0.1 µg; N=6		Group Dose 2 1 µg; N=6		Group Dose 3 10 µg; N=6		Group Placebo N=6	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
CD4 counts	Day 0	778	216	592	181	735	101	696	150
	Week 12	676	205	671	142	806	117	745	187
CD4 %	Day 0	37	5	36	6	35	7	37	4
	Week 12	37	4	33	1	35	9	35	5
CD8 counts	Day 0	815	282	728	262	896	294	730	275
	Week 12	702	289	983	291	1013	310	830	331
CD8 %	Day 0	38	6	44	8	41	8	39	7
	Week 12	37	5	48	9	42	9	37	9
CD4/CD8 ratio	Day 0	1.01	0.29	0.87	0.32	0.90	0.36	1.03	0.33
	Week 12	1.03	0.25	0.71	0.17	0.89	0.40	0.98	0.31
% NKp44L on CD4	Day 0	0.4	0.2	0.6	0.1	0.9	0.2	0.8	0.3
	Week 12	0.5	0.2	0.9	0.2	0.8	0.3	0.8	0.3
naive CD4	Day 0	39	14	25	9	31	12	37	3
	Week 12	35	9	28	10	31	10	39	6
Central Memory CD4	Day 0	28	8	30	9	28	5	30	5
	Week 12	26	6	31	9	28	4	28	9
Activation CD4 (HLA-DR⁺CD4⁺)	Day 0	11	3	19	5	18	7	16	7
	Week 12	14	4	21	5	13	6	14	6
Activation CD8 (HLA-DR⁺CD38⁺CD8⁺)	Day 0	8	7	12	7	12	3	16	14
	Week 12	10	5	20	16	11	4	16	17

Table 3: VAC-3S Primary Endpoint: Safety & Tolerability.

	Dose 1	N=6	Dose 2	N=6	Dose 3	N=6	All Doses	N=18	Placebo	N=7	Overall	N=25
	Event	Subject	Event	Subject	Event	Subject	Event	Subject	Event	Subject	Event	Subject
NSAEs	**23**	6	**36**	6	**25**	6	**84**	18	**39**	7	**123**	25
Grade1	**19**	6	**29**	6	**22**	6	**70**	18	**28**	7	**98**	25
Grade2	**4**	2	**7**	4	**3**	2	**14**	8	**9**	5	**23**	13
Grade3	**0**	0	**0**	0	**0**	0	**0**	0	**1**	1	**1**	1
Grade4	**0**	0	**0**	0	**0**	0	**0**	0	**1**	1	**1**	1
Leading to corrective treatment	***12***	*5*	***8***	*5*	***4***	*3*	***24***	*13*	***8***	*6*	***32***	*19*
Leading to treatment discontinuation	***0***	*0*	***0***	*0*	***0***	*0*	***0***	*0*	***1***	*1*	***1***	*1*
Related	**12**	5	**24**	6	**15**	6	**51**	17	**18**	5	**69**	22
Expected local	**7**	4	**18**	5	**10**	6	**35**	15	**12**	4	**47**	19
Erythema	***0***	*0*	***0***	*0*	***1***	*1*	***1***	*1*	***1***	*1*	***2***	*2*
Induration	***1***	*1*	***3***	*1*	***1***	*1*	***5***	*3*	***2***	*2*	***7***	*5*
Pain	***6***	*3*	***15***	*4*	***8***	*4*	***29***	*11*	***8***	*1*	***38***	*19*
Expected systemic	**3**	2	**3**	2	**4**	1	**10**	5	**3**	2	**13**	7
Asthenia/Pyrexia	***1***	*1*	***1***	*1*	***2***	*1*	***4***	*3*	***2***	*2*	***6***	*5*
Myalgia	***1***	*1*	***0***	*0*	***1***	*1*	***2***	*2*	***1***	*1*	***3***	*3*
Headache	***1***	*1*	***2***	*2*	***1***	*1*	***4***	*4*	***0***	*0*	***4***	*4*
Other	**2**	1	**3**	3	**1**	1	**6**	5	**3**	2	**9**	7
Not related	**11**	5	**12**	5	**10**	3	**33**	13	**21**	6	**54**	19

Conclusion & Perspectives

- VAC-3S vaccine has shown safety, evidence of immunogenicity at 10 µg x3 injections IM q4 weeks.
- Higher doses and booster revaccination are currently investigated.
- We hypothesize VAC-3S therapeutic effect would encompass the following clinical applications & benefits:

① Reconstruct immune homeostasis in patients with immunological failure receiving ART, i.e. patients who fail to achieve CD4 count > 350 c/mm³ or 500 c/mm³, or fail to increase CD4 count 50-100 c/mm³ following ART initiation.
② In a functional cure multi-therapeutic approach: "shield" the immune system and allow response leading to a host-mediated control of HIV-replication in the absence of ART.
③ Potentialization of therapeutic vaccines generating CTL response against the HIV reservoirs by improving CD4 helper function through immune reconstitution.

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