

Single DermaVir Patch Treatment of HIV+ Individuals Induces Long Lasting, High Magnitude and Broad HIV-Specific T Cell Responses

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Abstract

Background:

DermaVir Patch Immune Therapy has been developed to attenuate clinical disease in HIV-infected individuals by induction of long-lasting HIV-specific T cells. Preclinical studies on SIV-infected and naive macaques demonstrated that the potent T cell responses induced by DermaVir are associated with clinical benefit including viral load reduction and survival. Human data for safety and immunogenicity is reported here.

Methods:

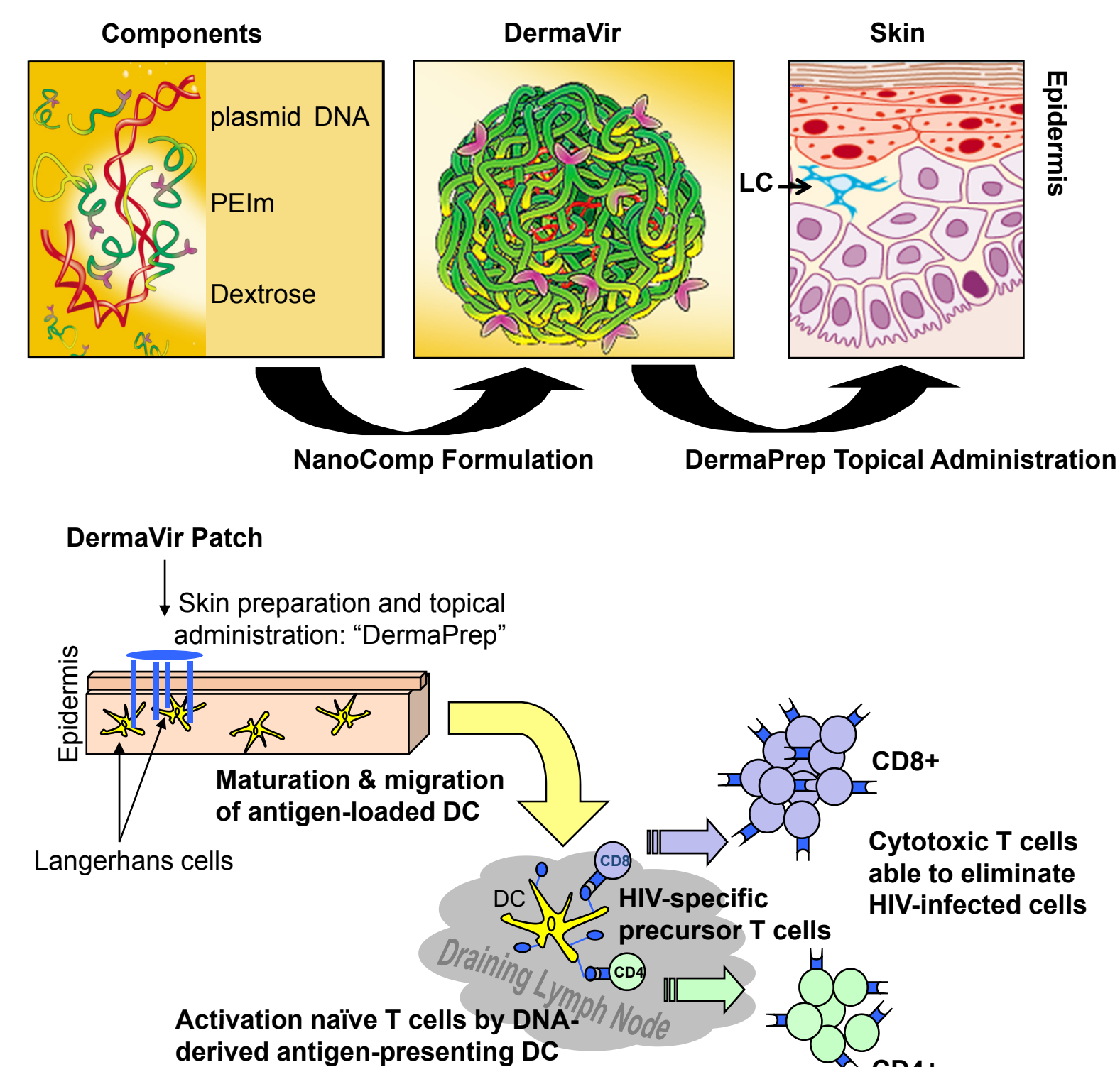
Nine HIV+ individuals on fully suppressive HAART were treated a single-time with increasing numbers of DermaVir Patches containing 0.1, 0.4 and 0.8 mg of multiple HIV genes encoding plasmid DNA, the active substance in DermaVir. HAART was continued during the one-year follow-up. Safety was assessed with standard clinical evaluation and laboratory assays, and dermatological analysis of skin reaction. HIV-specific (gag, tat, rev) effector T cells were measured by standard ELISPOT and T cell precursors with high proliferative capacity (PHPC) by cultured ELISPOT assay. Results are reported in number of spots/10⁶ cells.

Results:

All DermaVir Patch doses were safe and well tolerated. In all patient plasma HIV RNA, CD4 and CD8 counts remained in the pre-vaccination range throughout the study. The magnitude of gag-specific PHPC increased from <200 (baseline) to 2,300-4,000 at week 24 in the low dose, and to 2,500-21,500 and 3,000-37,000 at week 48 in the medium and high dose DermaVir Patch, respectively. The broadening of T cell responses was demonstrated by the induction of new rev- and tat-specific PHPC. These were mostly undetectable prior to immunization and increased up to 3,000-30,000 (rev) and 60-30,000 (tat) in the medium dose and up to 1,000-7,000 (rev) and 600-4,000 (tat) in the high dose, at day 28. In the medium dose cohort, for example, the average frequency of total PHPC and of effector T cells (shown in parenthesis) prior to vaccination were 124 (20); 28 days after vaccination 231,403 (720), and one year later 82,776 (n.d.), indicating >10 fold expansion of effectors and >100 fold of long-lived T cell precursors.

Conclusions: DermaVir Patch immunization was safe and well tolerated. The magnitude and breadth of HIV-specific T cell precursors significantly increased in all treated patients in a dose dependent manner, suggesting an excellent safety and immunogenicity profile in human subjects.

DermaVir Patch – Product & Mechanism of Action



GIHU004 Single DermaVir Patch treatment of HIV-infected subjects on fully suppressive ART

Study Population:

CD4 >300 cells/mm³ with nadir >250 cells/mm³
HIV-1 RNA PCR <50 copies/ml for >12 weeks

Cohort 1: 3 subjects

• Treatment: DermaVir Patches + continuous ART
• Dose: 2 DermaVir Patches (0.05 mg DNA per patch)

Cohort 2: 3 subjects

• Treatment: DermaVir Patches + continuous ART
• Dose: 4 DermaVir Patches (0.1 mg DNA per patch)

Cohort 3: 3 subjects

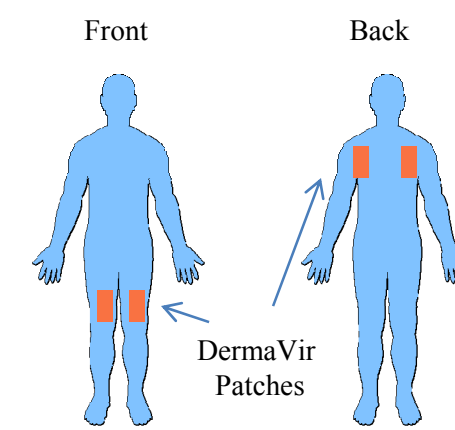
• Treatment: DermaVir Patches + continuous ART
• Dose: 8 DermaVir Patches (0.1 mg DNA per patch)

Summary of Results

Single DermaVir Patch treatments were safe and well tolerated.

There were no deaths, no serious adverse events, and no discontinuations from the study.

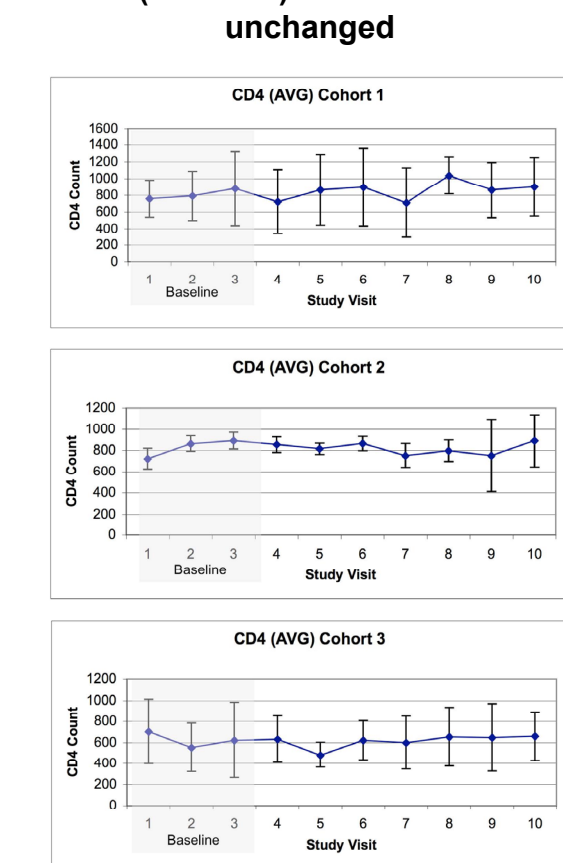
There was no unexpected or outstanding safety and tolerability related event.



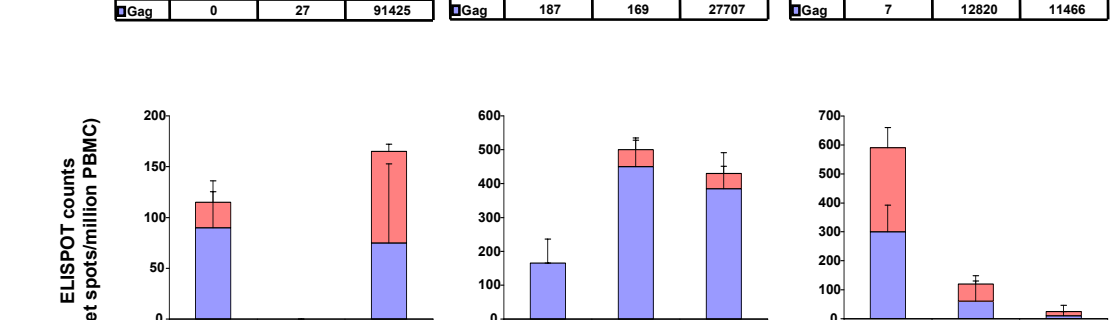
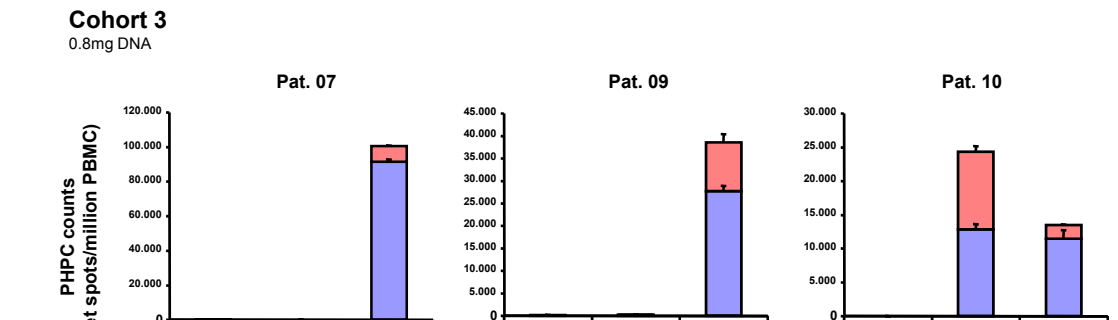
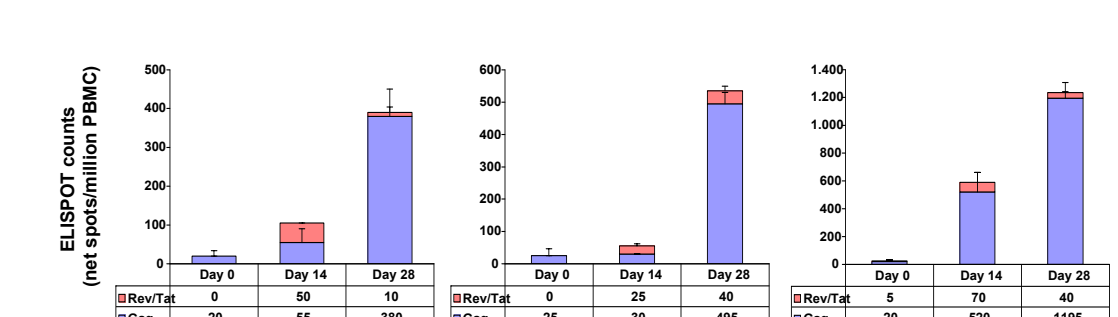
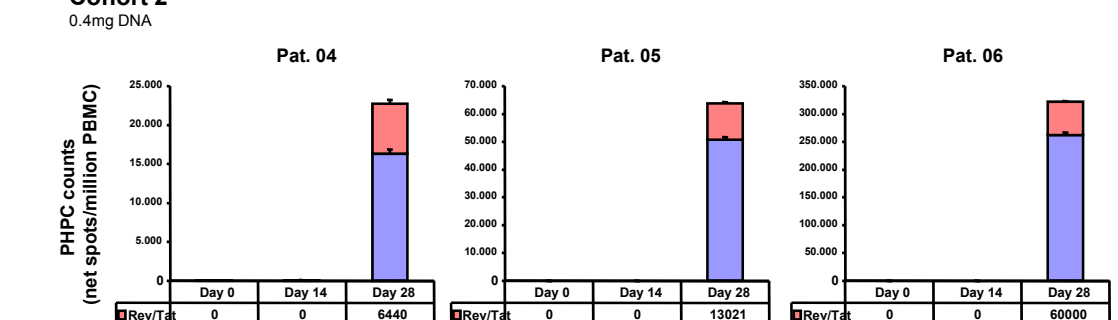
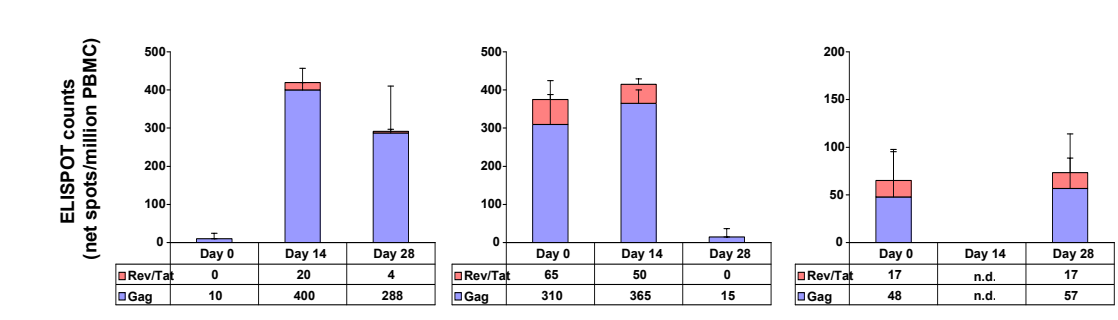
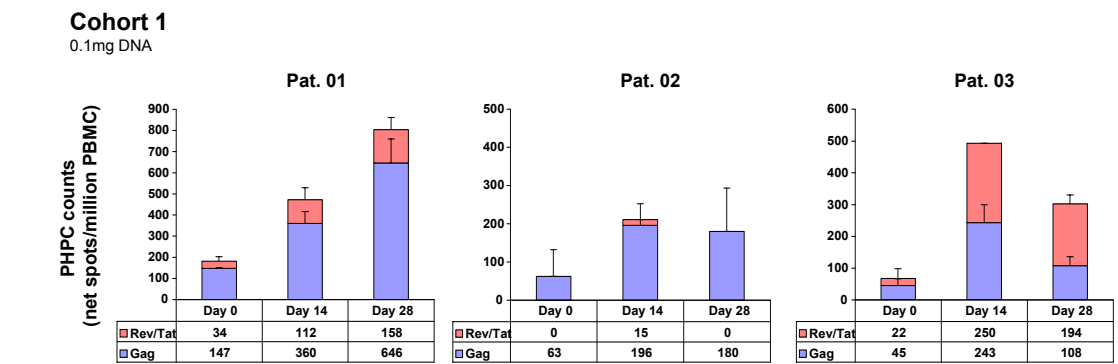
No HIV RNA blip was observed (ultra-sensitive PCR)

Coort	Date	Study Visit	HIV RNA Copies/ml
0.1 mg DNA	01/07/05	Pre-vacc	<5
	02/07/05	Day 0	<5
	03/07/05	Day 28	<5
	04/07/05	Pre-vacc	<5
	05/07/05	Day 0	<5
	06/07/05	Day 28	<5
0.1 mg DNA	02/07/05	Pre-vacc	<5
	03/07/05	Day 0	<5
	04/07/05	Day 28	<5
	05/07/05	Pre-vacc	<5
	06/07/05	Day 0	<5
	07/07/05	Day 28	<5
0.1 mg DNA	02/07/05	Pre-vacc	<5
	03/07/05	Day 0	<5
	04/07/05	Day 28	<5
	05/07/05	Pre-vacc	<5
	06/07/05	Day 0	<5
	07/07/05	Day 28	<5

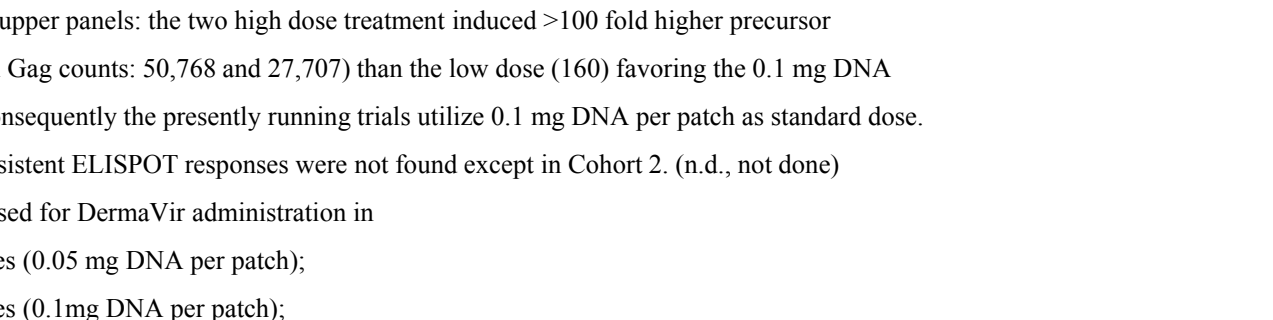
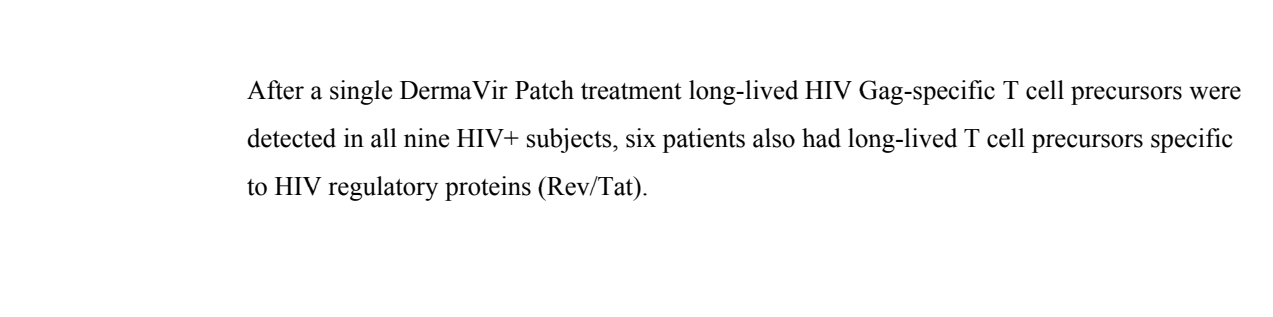
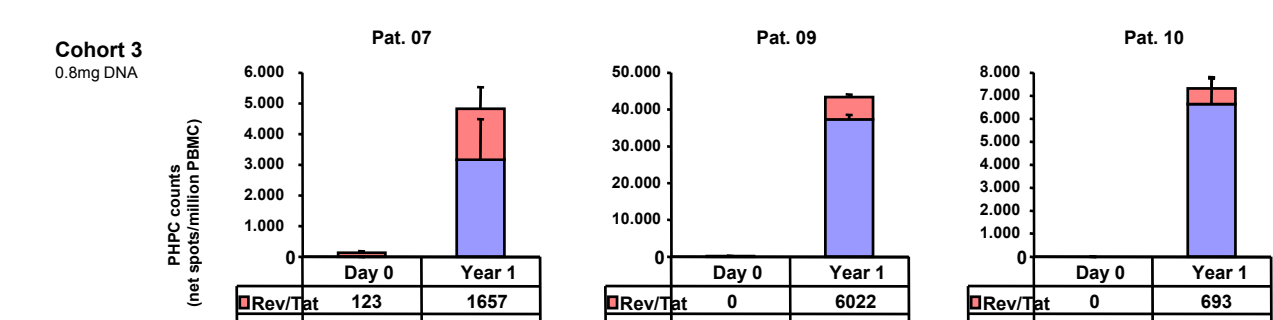
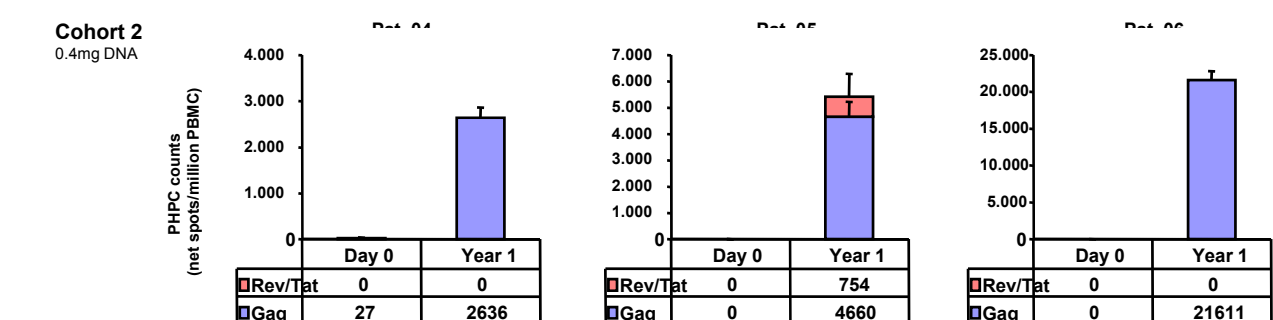
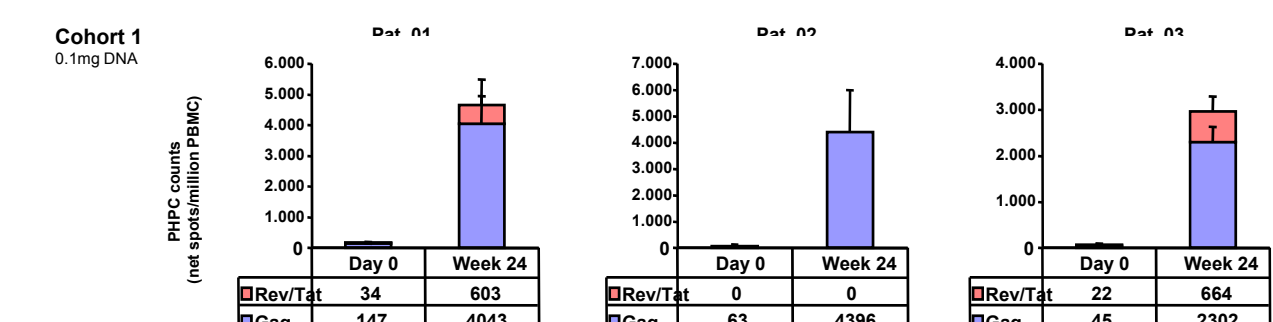
CD4 (and CD8) counts remained unchanged



Quantification of HIV-specific precursor T Cells with high proliferative capacity (PHPC) and effector T cells (ELISPOT) prior and after single DermaVir Patch treatment.



DermaVir-induced HIV-specific T cell precursors are present >1 year in patients treated by fully suppressive ART.



In each cohort the upper panels: the two high dose treatment induced >100 fold higher precursor frequency (median Gag counts: 50,768 and 27,707) than the low dose (160) favoring the 0.1 mg DNA dose per patch - consequently the presently running trials utilize 0.1 mg DNA per patch as standard dose.

Lower panels: consistent ELISPOT responses were not found except in Cohort 2 (n.d., not done)

The surface area used for DermaVir administration is

cohort 1 = 2 patches (0.05 mg DNA per patch);

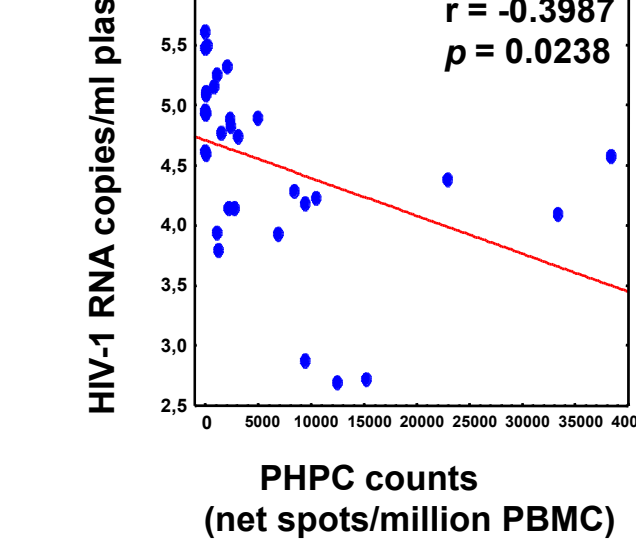
cohort 2 = 4 patches (0.1 mg DNA per patch);

cohort 3 = 8 patches (0.1 mg DNA per patch).

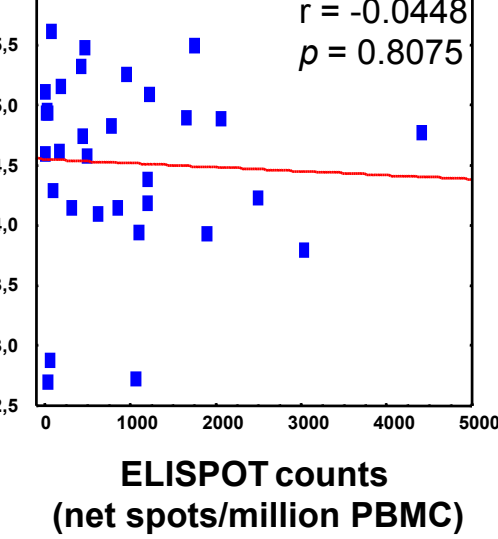
PHPC (Precursors with High Proliferative Capacity) assay

PHPC and ELISPOT assays were performed in response to peptides (15 amino acids in length with 11-amino acid overlap) representing the complete sequence of HIV-1 Consensus B Gag ($n = 123$, divided into three pools), Rev ($n = 27$, one pool) and Tat ($n = 23$, one pool) proteins. For ELISPOT assay, PBMC (1×10^5 cells/well) were stimulated for 24 hours with the corresponding HIV-1 peptide pool. For PHPC assay, PBMC (5×10^5 or 1×10^6 cells/well) were cultured with the corresponding HIV-1 peptide pool for 12 days. On days 3 and 7, recombinant human IL-2 was added. On day 12, cells were washed and tested (2.5×10^4 per well) in the same way as the ELISPOT in response to the corresponding HIV-1 peptide pool used for stimulation.

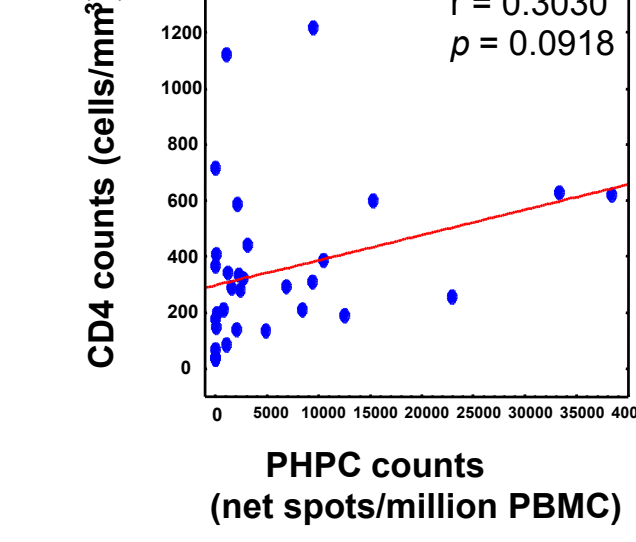
PHPC counts (net spots/million PBMC)



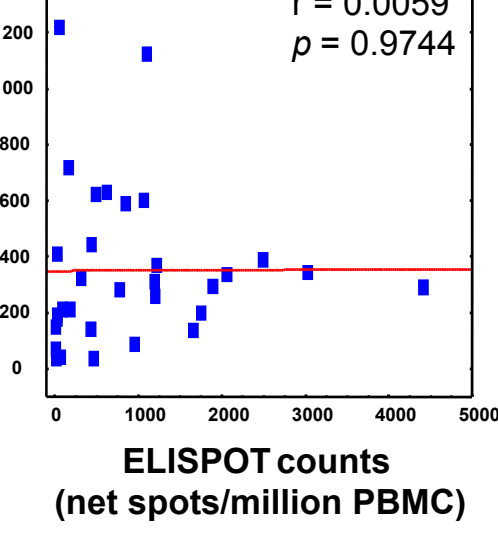
ELISPOT counts (net spots/million PBMC)



PHPC counts (net spots/million PBMC)



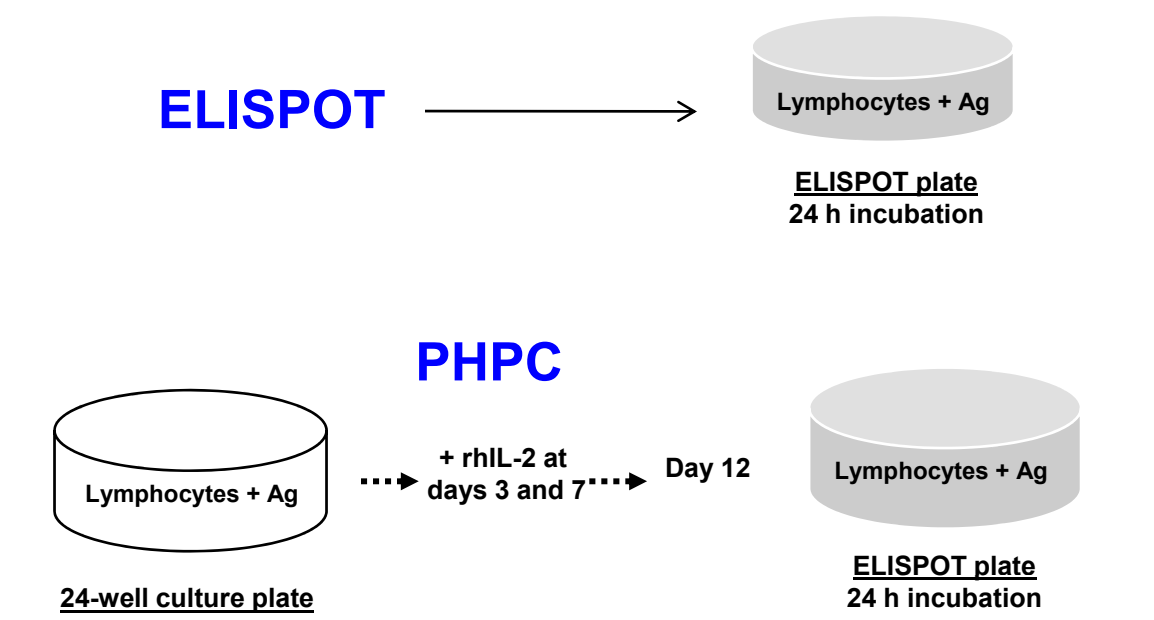
ELISPOT counts (net spots/million PBMC)



Synthetic peptides

Peptides (15 amino acids in length with 11-amino acid overlap) corresponded to the complete HIV-1 Consensus B Gag, Rev and Tat. Gag peptides ($n = 123$) were divided into three pools (41 peptides per pool), Rev peptides ($n = 27$) were in a single pool as well as Tat ($n = 23$).

Methods



Conclusion

Relevant immunogenicity assays:

- HIV Gag-specific ELISPOT counts do not correlate with plasma viral load.
 - ELISPOT might detect both short-lived effector and long-lived precursor T cells. However, the frequency of the long-lived precursors is very low in the peripheral blood, therefore the ELISPOT counts represent the terminally differentiated T cells capable to kill HIV-infected cells.
- HIV Gag-specific PHPC counts (Precursors with High Proliferative Capacity) negatively correlate with plasma viral load.
 - PHPC detects only precursor T cells which can proliferate upon peptide stimulation and mature to IFN- γ producing CTL. Short-lived effector cells are terminally differentiated and can not survive the 12-day culture period. Therefore, PHPC counts represent the frequency of HIV-specific precursors that after activation proliferate to CTL.
 - As marker of viral load it might be more relevant to investigate the precursors than the terminally differentiated T cells.

Immune responses induced by a single DermaVir Patch treatment of HIV+ individuals

- In all immunized patients HIV-specific precursor T cells were induced. These immune responses, different from ELISPOT responses, might correlate with viral load decreases.
- DermaVir Patch immunization using 0.4 and 0.8 mg DNA induced 10-100 fold more precursor T cell counts than using 0.1 mg DNA. In present trials the standard amount per one DermaVir Patch is 0.1 mg DNA.
- ELISPOT responses were induced in 5/9 patients. T cell responses measured by ELISPOT do not seem to have any relevance in either viral load suppression or protection against infection.
- One year after the single immunization HIV-specific precursor T cells were still present (10-1000 fold compared to baseline), albeit in lower frequency than 28 days after treatment, suggesting that the precursor T cells induced by this immunization have long half life.
- Single immunization broadened the specificity of HIV-specific precursor T cells. T cell responses were induced against both structural and regulatory proteins suggesting that the novel DNA plasmid in the product is a potent immunogen.