

Dendritic Cell-HIV Peptide Therapeutic Vaccination Is Safe and Immunogenic in HIV-infected Subjects with Virologic Suppression

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1. Introduction

DC-based vaccination has been used extensively in cancer trials as a means to increase cellular type, antigen-specific immunity. It has been uniformly safe in these subjects, with no hospitalizations or fatalities reported. Experience with this type of therapy in people with HIV is limited, with only 3 recently published trials involving fewer than 40 subjects.

The waning of HIV specific immunity during chronic infection most commonly results in a loss of immune control over viral replication and disease progression. DC-based vaccination is a promising modality for boosting HIV specific immunity and slowing or arresting HIV disease progression.

This phase I, prospective, randomized clinical trial for subjects on ART demonstrates the safety and feasibility of DC vaccination, using synthetic, HLA-A2 specific, HIV-1 peptides to load autologous, peripheral blood monocyte-derived DCs as a therapeutic vaccine in individuals with chronic HIV-1 infection on therapy.

4. Results

A. Clinical Characteristics

	mean (range)
Age	43.7 (25-65)
Black race	2/18 (11%)
Female gender	3/18 (17%)
CD4 nadir	274 (49-632)
Time since HIV diagnosis (months)	108 (48-180)

B. Comparison of Properties of Mature DCs Derived from Study Subjects with a Cohort of Normal Donors

	Mean (range)	Normal (n=5) Mean (range)
Yield	9.1 X 10 ⁷ (1.3 X 10 ⁷ -2.7 X 10 ⁸)	2.5 X 10 ⁸ (2.3-3.5 X 10 ⁸)
Viability	93% (69% - 99%)	90% (85%-95%)
Purity	83% (47% - 96%)	77% (75%-89%)
IL-12*	159 pg/ml (24-520 pg/ml)	478 ± 183 pg/ml

* IL-12 was performed by ELISA of supernatants from CD40L stimulated DCs

** Second plastic adherence required to increase purity

C. DCs from HIV+ Subjects Up-regulate Maturation Markers in a Manner Similar to Normal Donors



Data shown are from a representative HIV+ study subject

2. Schema



1. Leukapheresis

Subjects are randomized to receive vaccine either subcutaneously or intravenously and undergo a standard leukapheresis for 2.5 X blood volume.



2. Cells are placed in flasks and monocytes are separated by plastic adherence.



3. Immature DCs are derived from monocytes by culture for 6 days under cGM conditions in the presence of IL-4 and GM-CSF

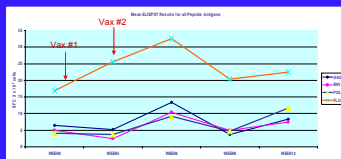
5. Vaccination (IV or SC)

18 subjects have each received 2 vaccinations, at weeks 1 and 4 following leukapheresis; *8 at low dose (1-3 million cells) *12 at high dose (5-10 million cells)

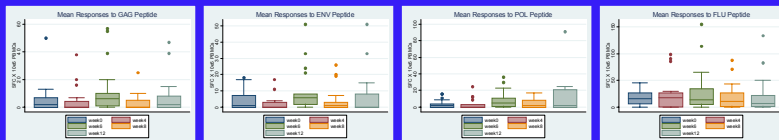
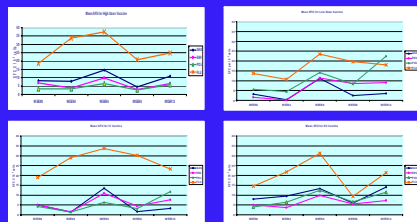


4. DCs are incubated for 24 hours in IL-1β, IL-6, TNF-α (each at 1:1 of 10ng/ml) and PGs-2 (1ug/ml), and pulsed for 2h, with synthetic, GMF-peptide peptides:
HIV-1 Gag: 386-394 VLASAMQV
HIV-1 Env: 134-142 KILPLVLT
HIV-1 Pol: 488-506 RAGLHSDY
H1A MP: 58-66 ILGLFVIL
Peptides chosen were HLA-A2 supertype, highly conserved, immunodominant epitopes.

D. Summary of IFN-γ, T-Cell Responses in Response to Vaccination measured by ELISPOT assay



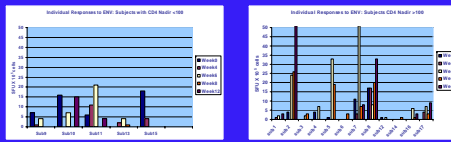
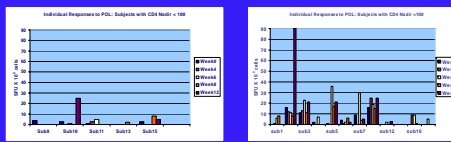
- Increased immunogenicity as measured by IFN-γ ELISPOT assay, for HIV and Flu vaccine peptides was seen following the second vaccination. The increase in T cell response showed a trend toward significance in this small group, specifically, gag, pol and flu at week 6 (p = 0.03, 0.06 and 0.10 respectively).
- Between groups, i.e. high dose vs. low dose and IV vs. SC, there were no major differences.



- Box plots, (above), show results, per peptide for the group as a whole and demonstrate that within each group there was considerable variability of response as indicated by the frequency of peptide-specific T cells in the peripheral circulation.

- The bar graphs (right – shown for Pol and Env only) demonstrate that there the greatest T-cell responses were in those individuals with higher CD4 nadirs (Env at week 8, p=0.028 and Pol at week 6, p=0.007). Also, there are certain individuals (2, 5 and 7) who are better immunologic responders than others.

- The frequencies for Flu-specific T cells were consistently higher pre- and post-vaccination than those for HIV-peptide specific T cells, possibly owing to the fact that these individuals had all received flu vaccination in the past.



3. Methods

- ELISPOT assays for IFN-γ were performed on 10⁵ PBMC/well in triplicate wells. PBMCs were incubated with 10⁴ peptide-pulsed, autologous DCs for 24 hours.
- Results (spot#/well) were read on a Zeiss ELISPOT reader.
- PBMCs mixed with un-pulsed DCs was used as background and subtracted from test wells.
- A positive response in ELISPOT assays was defined as the number of spots being greater by at least 2 Standard Deviations than background count and exceeding the background count by at least 20 spots/10⁵ PBMC.
- Two sided paired t-test statistic was used to compare means of triplicate wells at baseline (prior to vaccination) with means of triplicate wells at weeks 4, 6, 8 and 12 responses (following vaccination). Two sided two sample t-test statistic was used to compare means of test wells between high and low doses, and IV versus SC routes of administration. Repeated measurements ANOVA was used to test interactions between dose (high, low), route (IV, SC) and between subjects with low CD4 (<151) nadirs and those with higher ones (>237), for all peptides.

5. Conclusions

- DC vaccination was safe in all HIV-1+ subjects studied. One subject had an undocumented, grade 4 fever following vaccine #1, vaccine #2 was held. No significant adverse events were noted in the other 17 subjects.
- Yield, viability, purity and phenotype of DCs indicate that DCs obtained from HIV-infected subjects were comparable to DCs we generate from normal donors. Thus, DC-based vaccination could be a feasible strategy for HIV-1 infected subjects.
- DC vaccination increased the frequency of HIV-1, peptide-specific T cells in the peripheral circulation of study subjects. These T cells were responsive to synthetic HIV and Flu peptides ex vivo, as measured in IFN-γ ELISPOT assays.
- No significant differences in the frequency of T-cell responses were observed between the higher or lower DC dose and their SC or IV administration.
- Considerable inter-individual variability was observed in the frequency of HIV-1-peptide specific T cells detectable after vaccination in the peripheral circulation. Further study is needed to determine the cause of individual variation in immunologic responses to the DC-based vaccine.
- This pilot study demonstrates that DC-based vaccination is a promising therapy to improve immunologic responses against HIV in some individuals. Larger studies and broader antigen panels are needed to examine the true efficacy of this technique in the HIV-1 infected population.