mRNA-based dendritic cell vaccination induces potent antiviral T-cell responses in HIV-1 infected patients

Van Gulck E[1], Vlieghe E[2], Vekemans M[2], Van Tendeloo VFI[3], Van De Velde A[3], Smits E[3], Anguille S[3], Cools N[3], Goossens H[3], Mertens L[2], De Haes W[1], Wong J[4], Florence E[2], Vanham G[1], Berneman ZN[3]

1. Department of Biomedical Sciences, Unit of Virology, Institute of Tropical Medicine, Antwerp, Belgium; **2.** Department of Clinical Sciences, HIV and STD Unit, Institute of Tropical Medicine, Antwerp; Belgium; **3.** Center for Cell Therapy and Regenerative Medicine, Antwerp, Belgium; **4.** Department of Medicine, Massachusetts General Hospital, Boston, USA

Setting

Phasel/II therapeutic vaccination, based on autologous monocyte-derived dendritic cell (Mo-DC), electroporated with mRNA encoding HxB2 Gag, Tat, Rev and Nef in HIV-1 infected subjects under stable HAART.

Research question

Safety, feasibility and induced T cell responses: ELISPOT, proliferation, polyfunctionality and virus-suppressive activity.

Subjects and methods

Subjects (Table 1) : Six chronic HIV-1 patients with baseline CD4 T cell counts above 500 cells/µl, nadir CD4 T cell counts not below 300 cells/µl and plasma viral loads < 50 copies/ml for at least 3 months.

Vaccination scheme (Fig 1 A and B): Clinical grade DC vaccines were prepared from Mo after leukapheresis, CD14 selection by Clinimacs and in vitro differentiation (GM-CSF + IL-4). Mo-DC were electroporated (EP) with codon optimized HxB-2 *gag* mRNA or *tatrevnef* mRNA. Each vaccine of 10 x 10^6 mature EP DC was injected 50% intradermally and 50% subcutaneously on four occasions (V1-V4). The general scheme is shown in A; the phenotypic characteristics of the DC are shown in B.

Figure 1A. DIVA trial set up

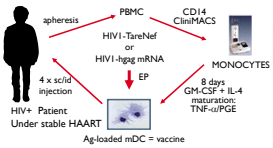
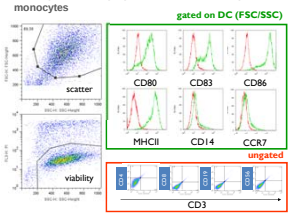


Figure 1B. Quality control of GMP-grade RNA-electroporated DC prepared from CliniMACS-purified monocytes



Safety: was evaluated by careful observation of the patients. Blood was drawn for hematogy, chemistry, liver en kidney function, HIV-1 plasma viral load, CD4+ and CD8+ T-cell counts, anti-nuclear antibodies and rheumatoid factor.

Immune monitoring: Blood was drawn 3 weeks before vaccination (time T1), 1 week after the second vaccination (T2), 1 (T3) and 6 weeks (T4) after the last vaccination. PBMC were cryo-preserved.

1.IFN-γELISPOT (Fig 2): Thawed PBMC were stimulated overnight with HxB2 peptide pools (12 Gag, 2 Tat, 5 Nef, 3 Rev pools in the x-axis). The number of IFN-γ spot forming cells (SFC) per million PBMC is shown; the color of the graphs corresponds to the time points indicated on the time axis on top. Increased breadth of IFN-γ response is indicated by a star; w, number of weeks in relation to the first DC vaccination (V1).

2.T cell proliferation (Fig 3): the ^{3}H-thymidine uptake assay was performed on thawed PBMC, cultured for 6 days with HIV-1 peptide pools that scored positive in IFN-γ ELISPOT. T-cell proliferation was reported as stimulation index (SI).

3.Polyfunctionality (Fig 4): Thawed PBMC were stimulated in the presence of brefeldin A with those peptide pools that scored positive in ELISPOT. After stimulation for 6 hr, cells were surface stained with anti-CD3, anti-CD4, anti-CD8 and anti-CD69 and intra-cellularly with anti-CD107a, anti-IFN-γ, anti-TNF-α and anti-IL-2. Each slice of the pie represents the percentage within CD4+ and CD8+ activated T-cells expressing a designated number of markers within 1 cell. Values indicated by a star represent an increase during and after DC vaccination (T2, T3, T4) by at least 50% as compared to pre-vaccination (T1).

4.Virus suppression assay (Fig 5): The set-up is depicted in A; results in B. Purified activated CD4+ T-cells were obtained by anti-CD3/CD8 bifunctional Ab stimulation, infected with 0.01 MOI of HIV-1 IIIB and cultured alone or in the presence of equal numbers of autologous CD8+ T- cells (obtained by anti-CD3/CD4 Ab stimulation). HIV-1 p24 production was measured on day 13 in ELISA. The percentage inhibition of viral production in CD4+/CD8+ cell co-cultures as compared to CD4+ T-cells alone is represented on the y-axis. Viral inhibition was assessed in 5 patients (represented with different symbols).

Results

SAFETY AND FEASIBILITY (Table 1): Only local side effects. No feasibility problems. Stable clinical, viral and other parameters.

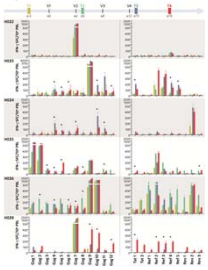
Table 1. Patient characteristics

UPN	Demographic data				Nadir	CD4+ T cell count/µL						Plasma viral load (copies/mL)					
	Sex	Age (yr)	HIV+ (yr)	HAART		V1 Pre (T1)	V1 +1w	V2 +1w (T2)	V3 +1w (T3)	V4 +1w (T4)	V4 Max (T4)	V1 Pre (T1)	V1 +1w	V2 +1w (T2)	V3 +1w (T3)	V4 +1w (T4)	V4 Max (T4)
H022	♂	46	15	abacavir + lamivudine + zidovudine; tenofovir	281	842	689	732	782	812	856	<50	<50	87	<50	<50	<50
H023	♂	42	17	nevirapine; lamivudine + zidovudine	307	750	901	573	537	581	736	<50	<50	<50	<50	<50	<50
H024	♂	39	4	abacavir + lamivudine + zidovudine	315	436	504	344	424	553	470	<50	<50	<50	<50	<50	<50
H025	♂	35	6	efavirenz + emtricitabine + tenofovir	497	497	440	576	598	462	479	<50	<50	<50	<50	<50	<50
H026	♂	46	3	abacavir + lamivudine + zidovudine	1039	1173	1201	1092	1111	1126	1102	<50	<50	<50	<50	<50	<50
H028	♂	45	5	nevirapine; emtricitabine + tenofovir	400	570	492	482	522	560	418	<50	56	<50	<50	63	<50

NOTE. UPN, unique patient number; Age, age at start of DC vaccination; HIV+, years of HIV-1 infection prior to start of DC vaccination; HAART, antiretroviral therapy during DC vaccination; Nadir, lowest CD4+ T cell count before HAART; V, DC vaccination; w, week. Plasma viral load was determined using the Roche Amplicor HIV-1 Monitor™ UltraSensitive assay.

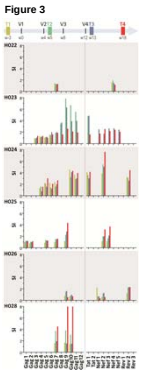
ELISPOT (Fig 2):
- Magnitude of Gag responses increased after vaccination in all patients (p <0.001).
- Breadth of Gag responses increased in 5/6 (p = 0.01).
- Tat-Rev-Nef responses showed less significant changes.

Figure 2



PROLIFERATION (Fig 3):
Increase in SI when all peptide pools were considered (p < 0.001).

Figure 3



POLYFUNCTIONALTY (Fig 4):
Variable picture, but:
- Overall % of T cells with > 2 functions increased by 50 % in 3/6 patients.
- CD4 T polyfunctionality increased in 2/6 between T1 and T4.
- CD8 T polyfunctionality increased in 3/6 between T1 and T4.

CD8 T VIRUS SUPPRESSION CAPACITY against HIV-1 IIIb (Fig 5):
- Very obvious increase in 3/5 patients (H024, H026 and H028).
- Slight increase in H025 (but already high at T1)
- Variable in H022.
- This picture was less consistent with other viruses.

Figure 4

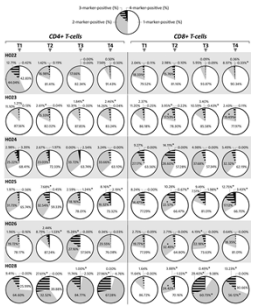
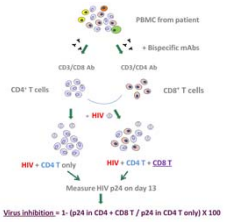
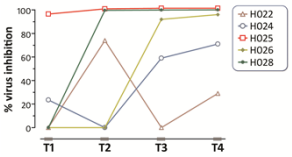


Figure 5A. Set-up virus inhibition assay



Virus inhibition = 1- (p24 in CD4 + CD8 T / p24 in CD4 T only) X 100

Figure 5B. Results virus inhibition assay



INTERRELATION BETWEEN VARIOUS RESPONSES:
- Virus suppressive activity at T4 correlated with Gag ELISPOT magnitude (R = 0.57 p < 0.05) and breadth (R = 0.56 p < 0.05). No correlation with Tat-Rev-Nef.
- Polyfunctionality also correlated with ELISPOT and proliferative responses.

Discussion

1. Gag and TatRevNef mRNA loaded autologous DC vaccination is safe, feasible and immunogenic in HIV-infected subjects under stable HAART.
2. CD8 T cell mediated virus suppression activity against a vaccine-related strain can be induced and correlates with Gag specific T cell responses.
3. Breadth and polyfunctionality of the responses should be improved by including more universal sequences (e.g. conserved or mosaic) and more powerful immune enhancers (e.g. IL-21 or PD1 blocking).

Institute of Tropical Medicine, Antwerp
Nationalestraat 155, B-2000 Antwerp, Belgium
Tel. +32-3-247.62.28 Fax. +32-3-247.63.33
gvanham@itg.be - www.itg.be

Presented at the IAS Pre-Conference Symposium "Towards an HIV Cure", 20 & 21 July 2012