

EFFECT OF ASSOCIATING A CYTOSTATIC DRUG + HAART AND HOLDING THE CYTOSTATIC DRUG AFTER STI AND A DEFINITIVE INTERRUPTION OF HAART ON HIV-1-SPECIFIC IMMUNE RESPONSES

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BACKGROUND

- **Cycles of Structured Treatment Interruption (STI) may be useful to partially control viral replication in a low percentage (about 20%) of chronic HIV-1 infected patients.**
- **Cytostatic drugs may further increase the proportion of responding patients.**
- **It has been hypothesized that hydroxyurea (HU) could help to control viral replication, probably by its cytostatic effect on CD4+ T lymphocytes. Nevertheless, the effects of HU on the HIV-1-specific immune responses are not well known.**

OBJECTIVES

To study the effect of associating hydroxyurea (HU) plus antiretroviral therapy (ART) versus ART alone after 5 cycles of STI and a definitive interruption of antiretroviral treatment but holding HU, on HIV-1-specific neutralizing antibodies activity (NA), CTL and helper responses in chronic HIV-1-infected patients.

MATERIAL AND METHODS I

- **Study design and patients:**

Twenty chronic HIV-1-infected patients (CD4>500/mm³ and VL>5000 c/ml) treated with D4T+DDI+IND for 52 weeks and viral load below 20 c/ml for at least 32 weeks were randomized to receive D4T+DDI+IND+HU (HU group) (n=10) vs D4T+DDI+IND (ART group) (n=10) during 6 months. Thereafter, five consecutive short cycles of STI were undertaken separated by periods of 2 months with the same triple ART (which was reintroduced when VL increased above 200 c/ml in the 1st stop, after 2 weeks in the 2nd, 3rd and 4th stops, and after VL reached a set-point after the 5th stop). HU was discontinued during the periods off ART in the 1st, 2nd and 3rd stops, and maintained in the 4th and 5th (and last) stops.

- **Viral load:**

Plasma HIV-RNA copy levels were evaluated using commercially available AMPLICOR (Roche Diagnostic Systems) quantitative RT-PCR. Samples with less than 200 c/ml were retested using Ultra Direct Assay with a limit of quantitation of 20 c/ml.

- **Neutralizing activity assays:**

PBMC from healthy blood donors were isolated by Ficoll-Hipaque centrifugation and stimulated for 48 hours with PHA (3µg/ml)(Sigma-Aldrich, Steinheim, Germany) and rIL2 (100U/ml) (Amersham, Buckinghamshire, UK). Two hundred thousands activated PBMC (resuspended in 75 µl of RPMI 1640)(Sigma, St. Louis, MO) were added to 96 microplate wells. Seventy-five µl of a primary virus dilution [D50 adjusted to 25 for HIV#45 (amphotropic R3, R5, X4 strain that uses CCR3, CCR5, and CXCR4 as coreceptors) and to 16.7 for HIV#40 (R5 strain that uses CCR5 as its coreceptor) was added to 75 µl of serial dilutions (4-folds) of serum samples, incubated for 1 hour, and added to the activated PBMC. Cultures were incubated for 2 hours, washed and resuspended in PHA and IL2-containing medium. HIV-1 p24 Ag in the supernatants was calculated relative to a non-treated control.

MATERIAL AND METHODS II

- **Lymphocyte proliferation assays:**

PBMC were cultured in protein-free medium X-VIVO 10 at 2×10^6 in 7 days assays in 96 round-bottomed plates in the absence or presence of polyclonal stimulus (PWM) or recombinant p24 HIV-1 protein (5 μ g/ml). Incorporation of tritium-labelled thymidine was assessed for the last 18 h of culture. Results are expressed as Stimulation Index (SI): cpm with stimulus/ cpm without stimulus. Positive antigen-specific responses were defined as more than 3000 cpm and SI greater than 3.

- **ELISPOT assay:**

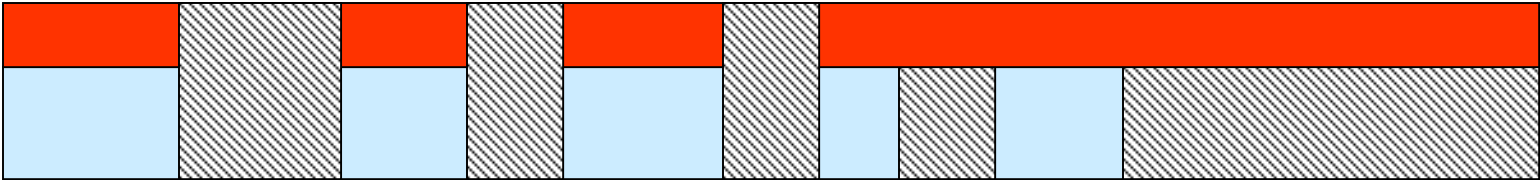
An epitope-specific ELISPOT assay was used to measure antigen-induced IFN- γ release from CD8 T-cells. A mean of 16 (range: 3-27) different HLA class I-restricted synthetic peptides from gag, pol, env and nef proteins were tested in each individual. Ninety-six well microtiter plates were coated overnight with a mAb specific to human IFN- γ (mAb 1-D1K, Mabtech, Sweden). PBMC resuspended in RPMI plus 10% FCS were plated in the presence of different peptides at 4 μ M and incubated overnight at 37°C, 5% CO₂. Plates were developed using biotinylated anti-human IFN- γ , streptavidin-bound phosphatase alkaline conjugate and chromogenic substrate (BioRad). Spots forming cells (SFC) were enumerated on a dissecting microscope. After subtracting background counts obtained with PBMC with medium alone, results were normalized to SFC/10⁶ PBMC.

PATIENTS AND METHODS

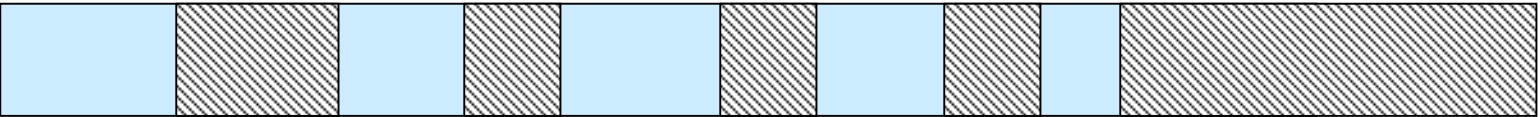
EARTH-2 STUDY
>5,000 c/ml
>500 CD4+

Hydroxyurea

No therapy



d4T+ddI+IDV



-74

-24

0

10 12

20 22

30 32

40

WEEK

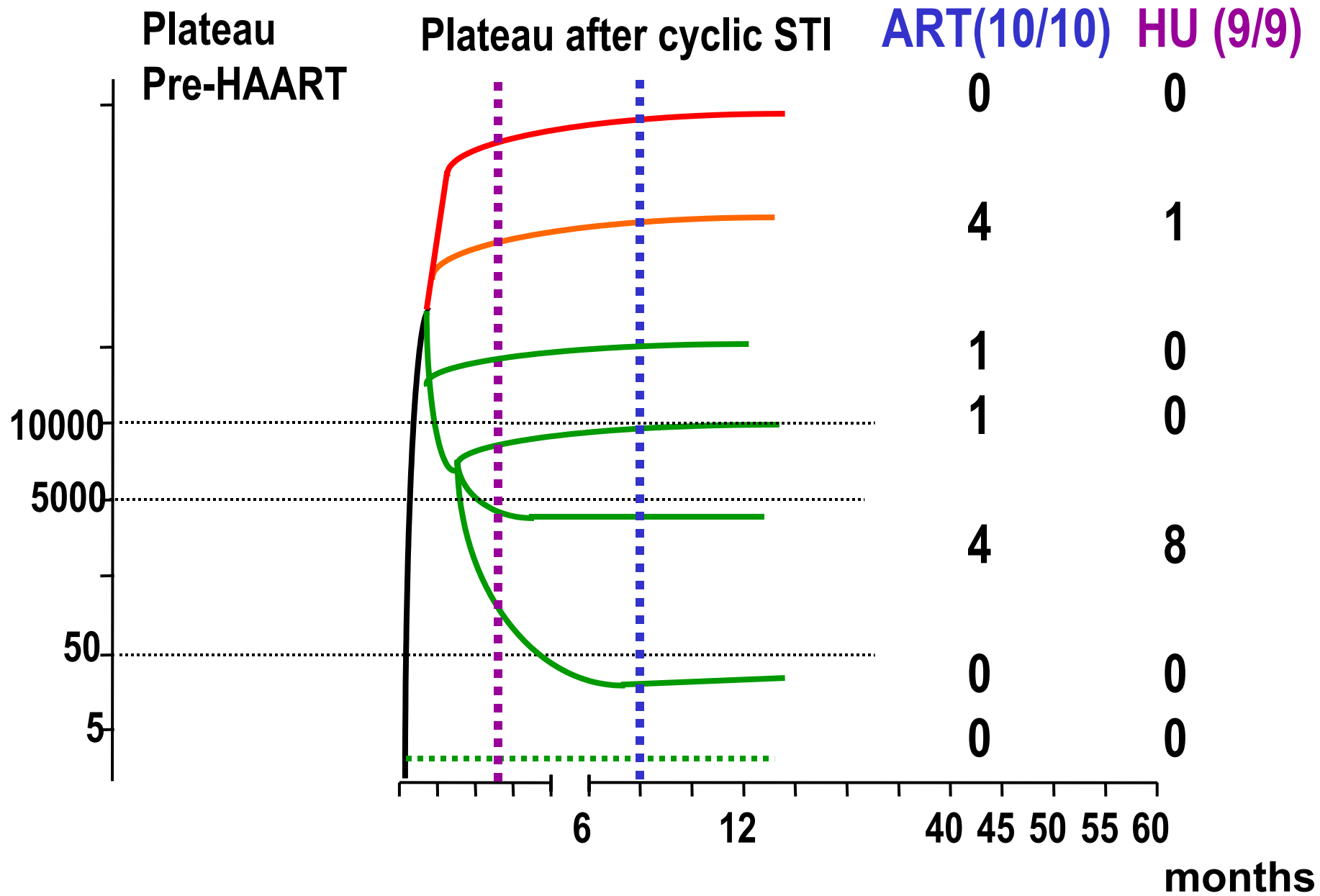
↑
1st stop

↑
2nd

↑
3rd

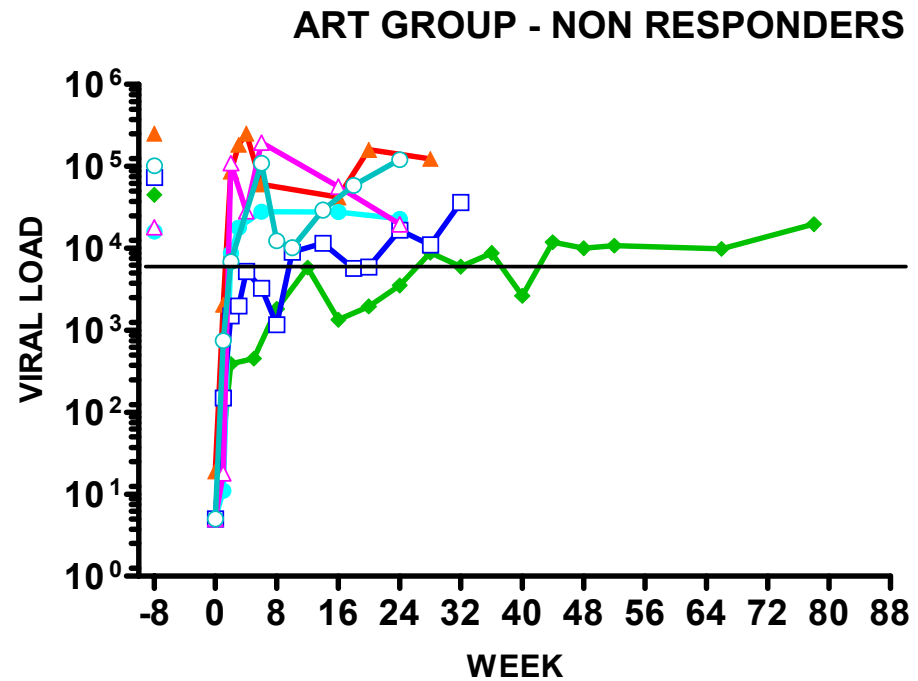
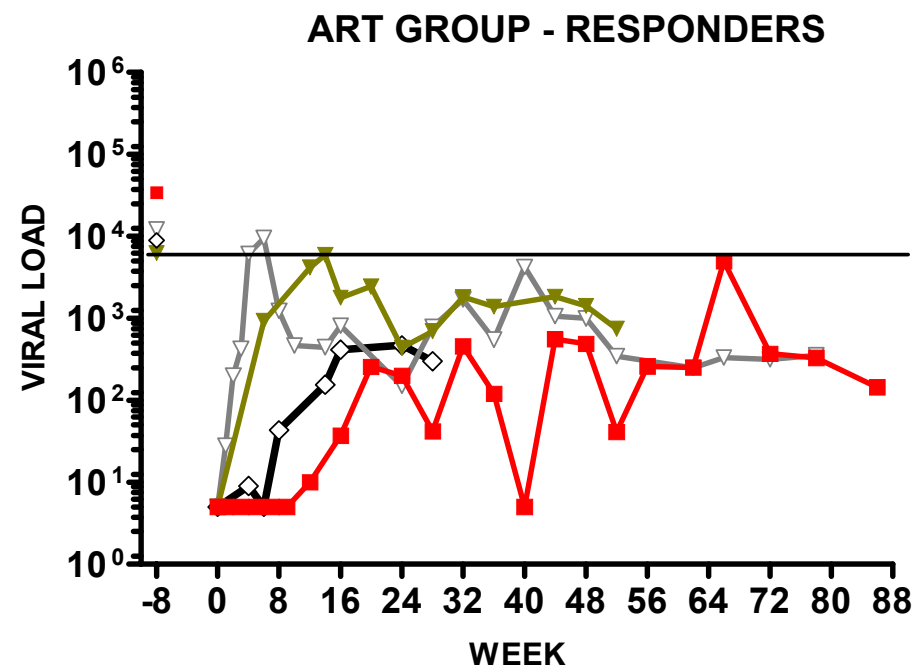
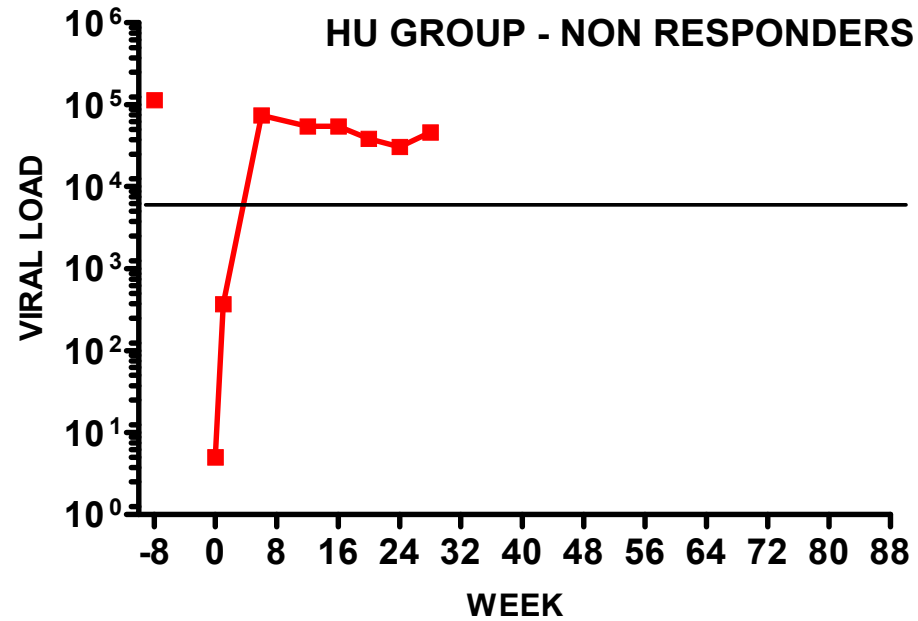
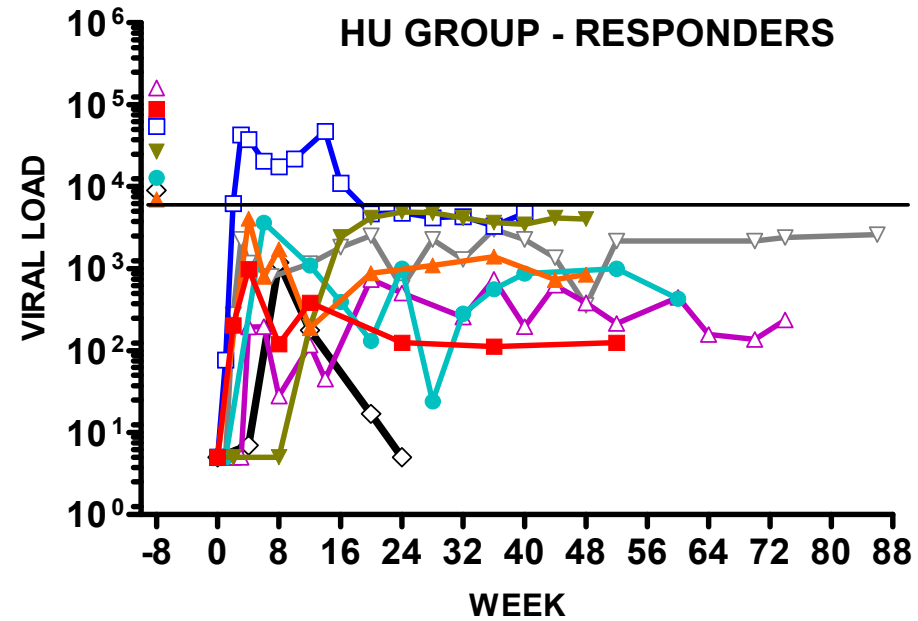
↑
4th

↑
5th and last stops

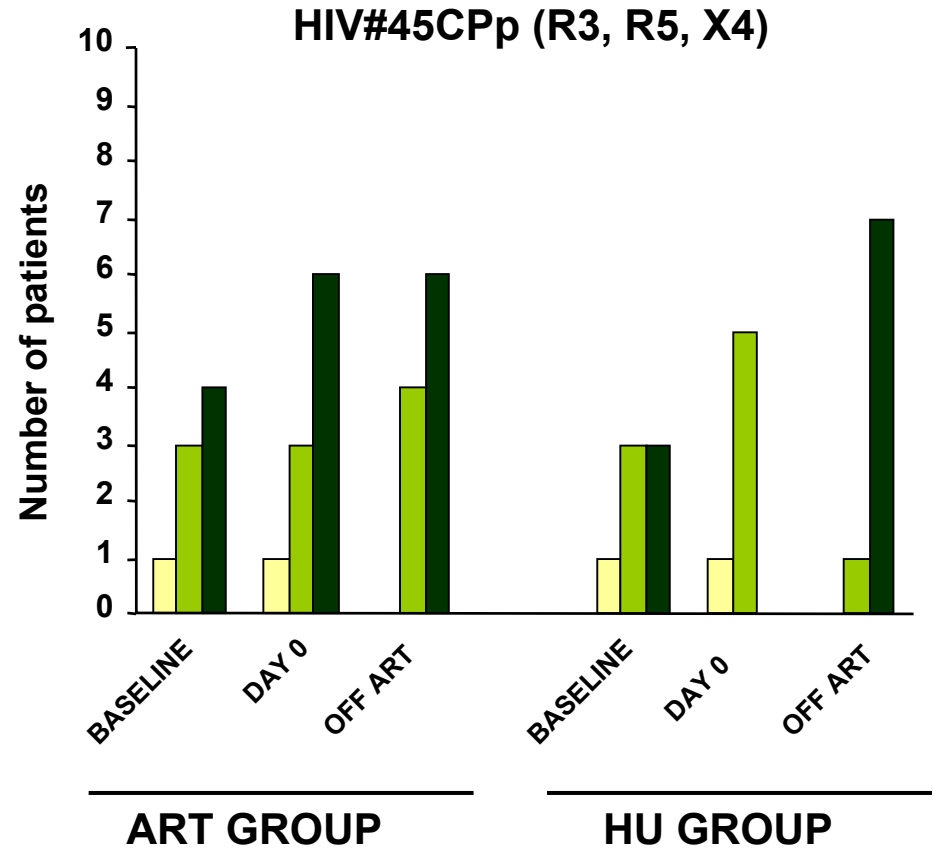
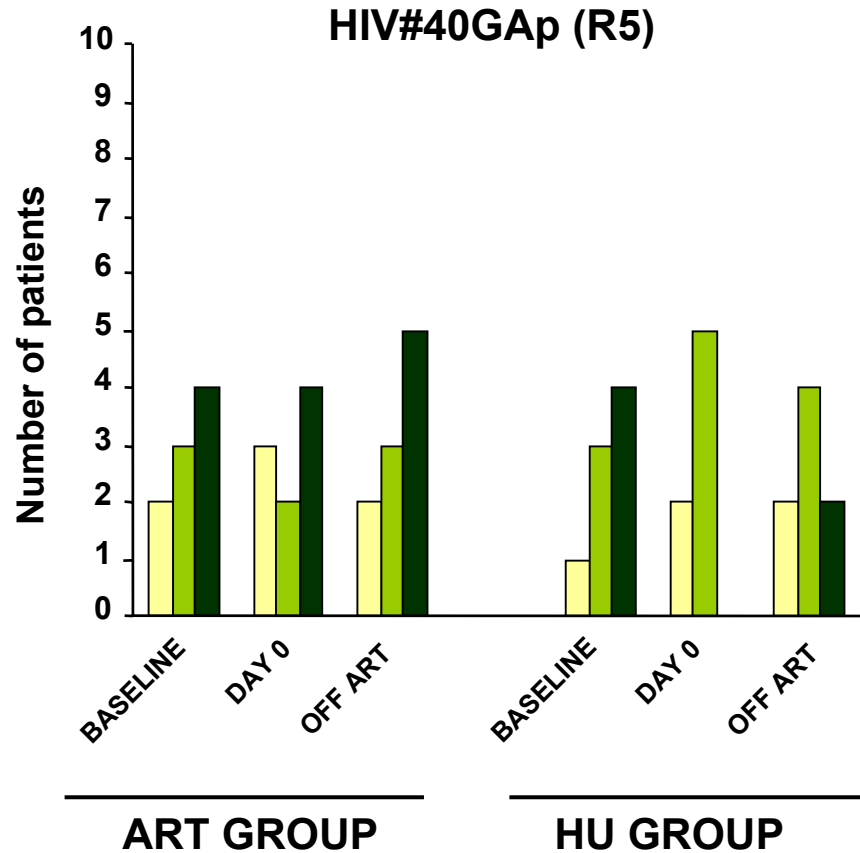


█ = ARV





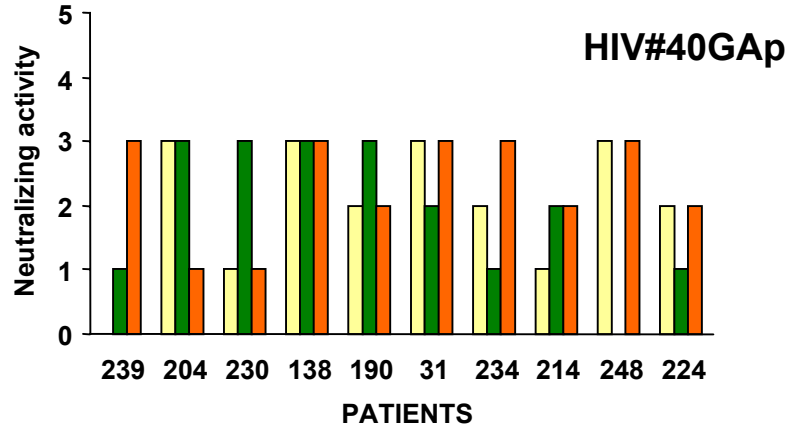
NEUTRALIZING ACTIVITY



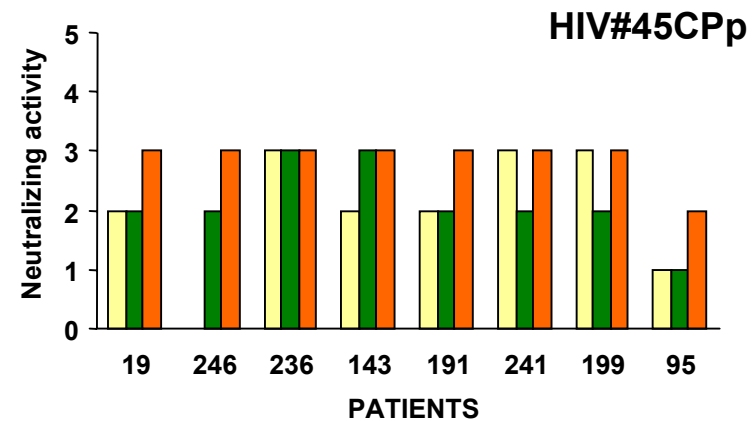
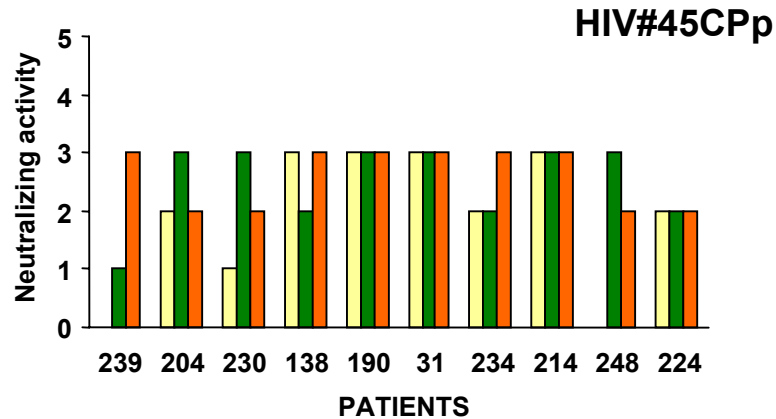
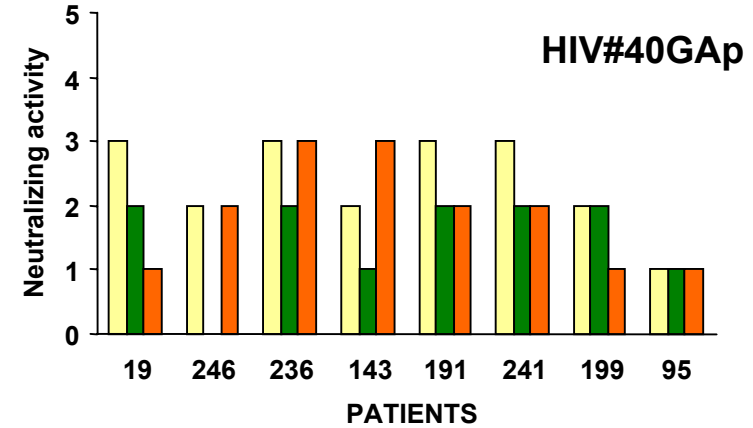
Neutralization at dilution <1/30
 IC50 at 1/30
 IC50 at 1/120

NEUTRALIZING ACTIVITY

ART GROUP



HU GROUP



Neutralizing activity: 1 at <math><1/30</math>, 2= IC50 at 1/30 and 3= IC50 at 1/120

Baseline
 Day 0
 OFF ART

CD4 PROLIFERATIVE RESPONSE TO HIV-1 ANTIGENS

Proportion of patients showing a SI > 3 in PBMC proliferative assays against recombinant HIV-1 p24.

	Day 0	OFF ART >w40
	3/19	17/19
ART group	1/10	9/10
HU group	2/9	8/9

HIV-1-SPECIFIC CTL RESPONSES

- **Total SFC/10⁶ PBMC (median and range)**

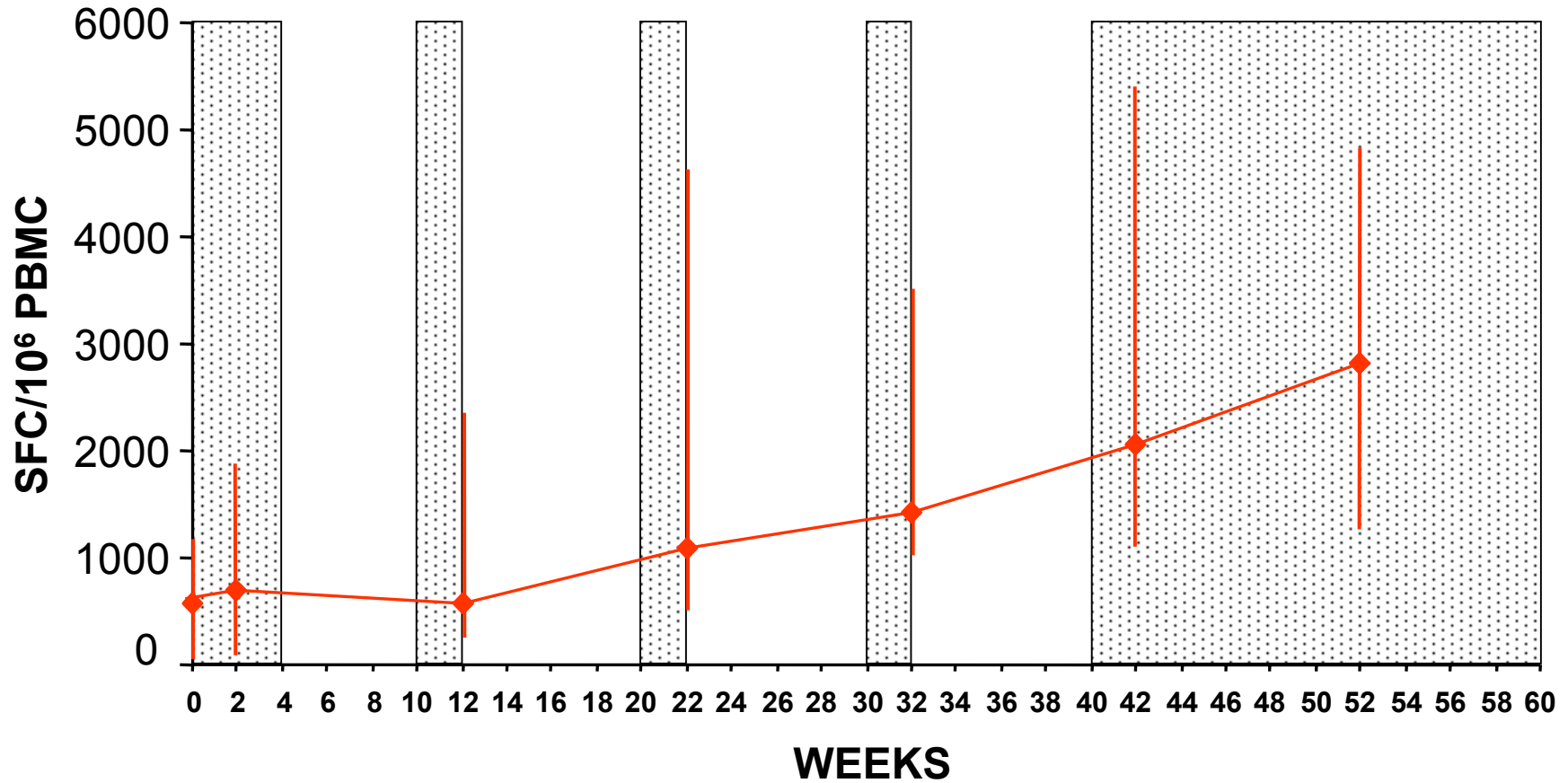
Day 0	w2	w12	w22	w32	w42	w52
570 (25-1080)	698 (72-1938)	582 (242-2294)	1080 (451-4664)	1429 (910-3281)	2071 (712-5619)	2814 * (813-4658)

* p < 0.0001

- **Number of epitopes recognized (median and range)**

Day 0	OFF ART > w42
3 (0 - 8)	6 (4 -16)

HIV-1-SPECIFIC CTL RESPONSE

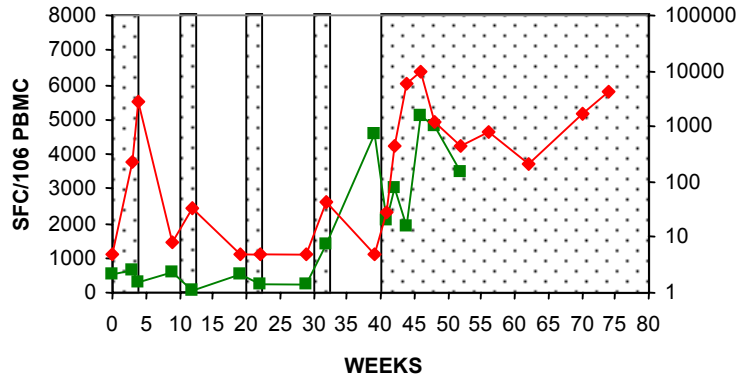


□ ON ART ▨ OFF ART

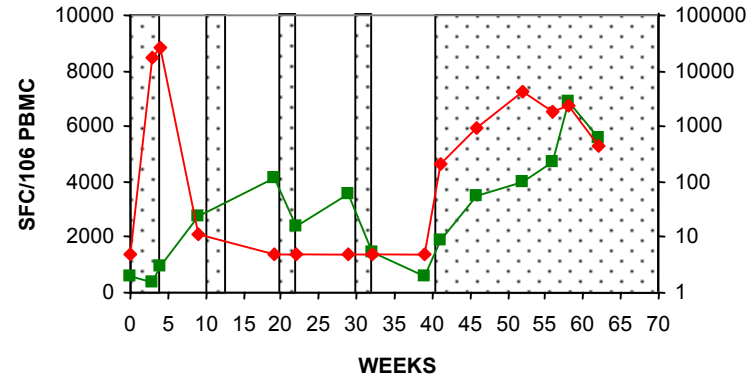
—◆— Median SFC/10⁶ PBMC

RESPONDER PATIENTS - ART GROUP

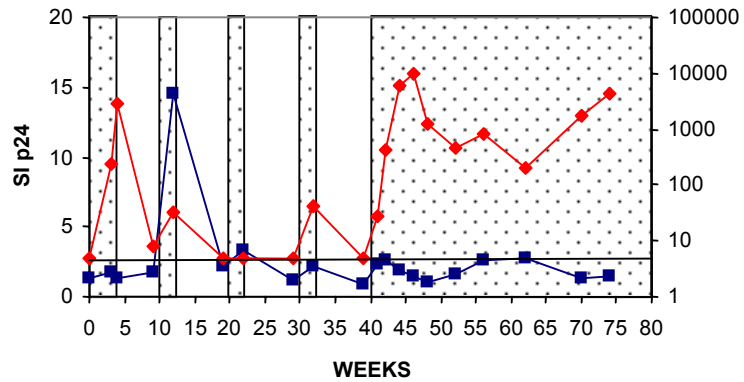
239-ART



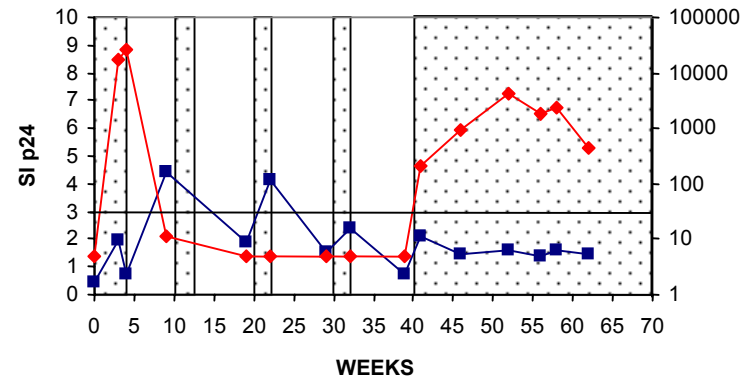
204-ART



239-ART



204-ART



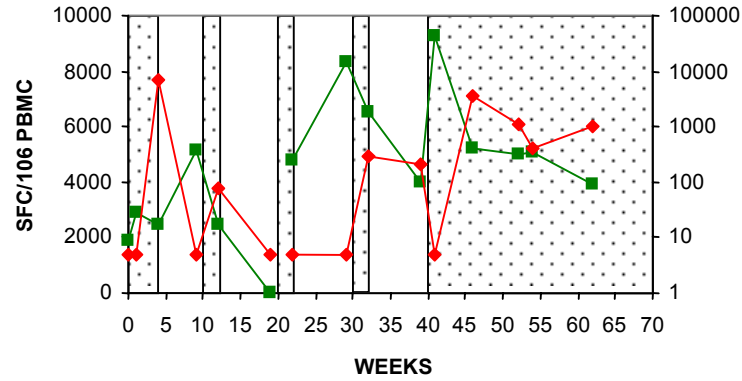
—■— TOTAL SFC/10⁶ PBMC

—■— VL

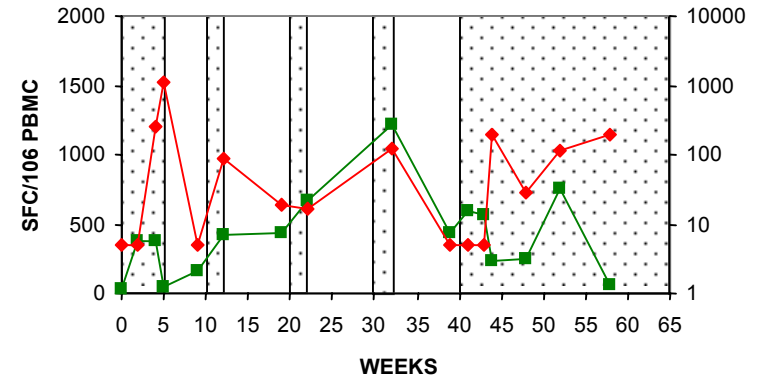
—■— SI p24

RESPONDER PATIENTS - HU GROUP

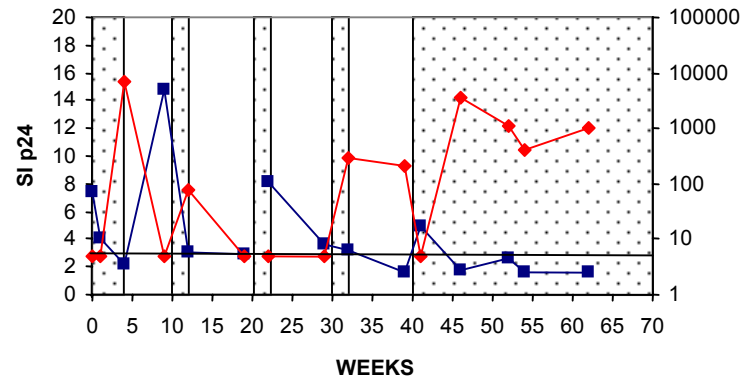
236-HU



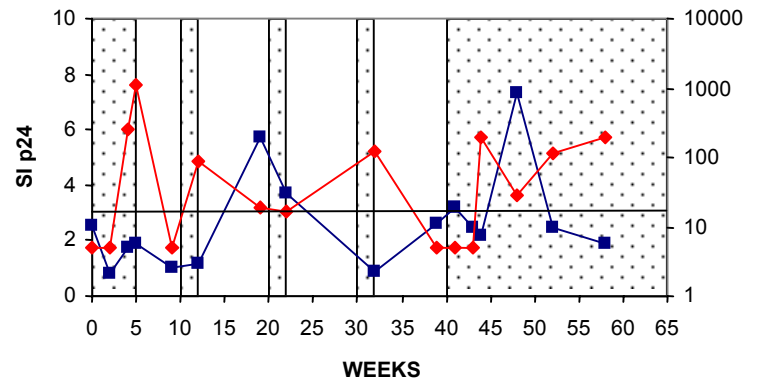
246-HU



236-HU



246-HU



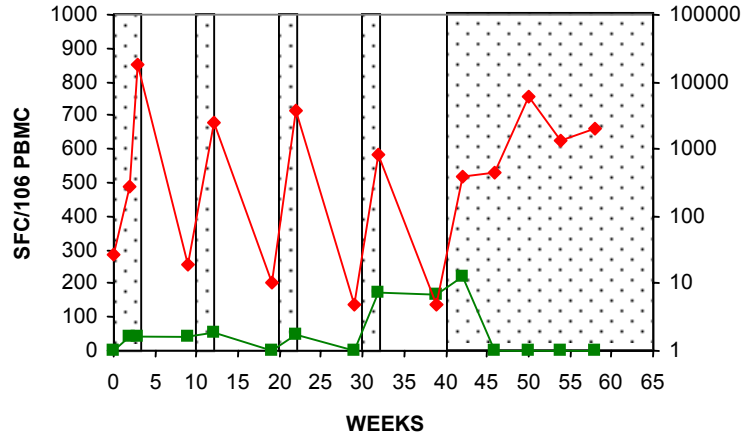
—■— TOTAL SFC/10⁶ PBMC

—■— VL

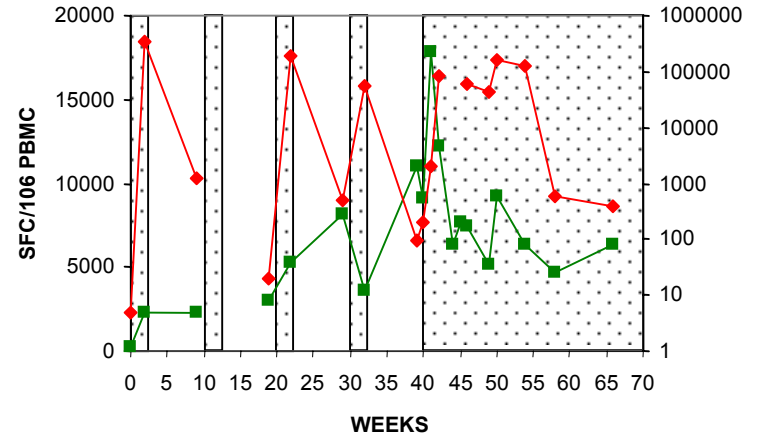
—■— SI p24

NON RESPONDER PATIENTS - ART GROUP

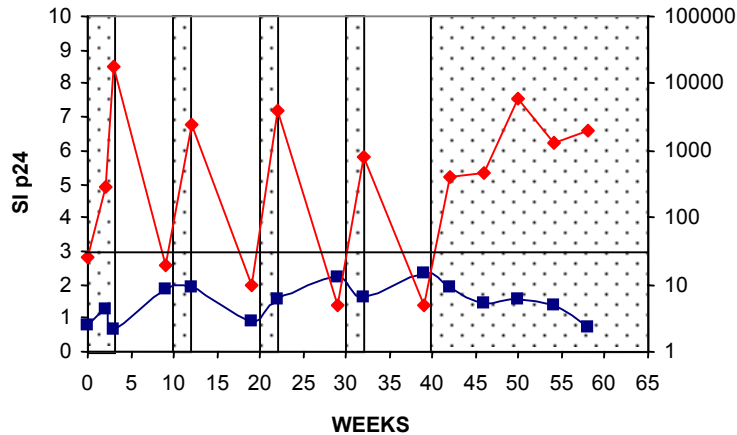
190-ART



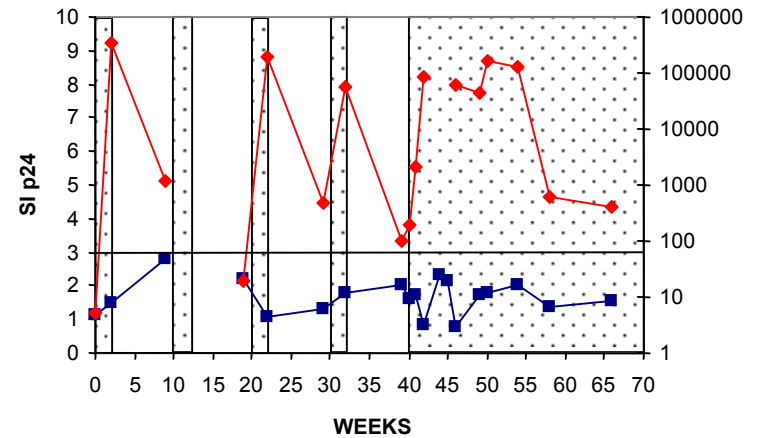
234-ART



190-ART



234-ART



—■— TOTAL SFC/10⁶ PBMC

—◆— VL

—■— SI p24

HIV-1-SPECIFIC IMMUNE RESPONSES

ART group (n=10)

OFF ART > w40

Patient	N Ab		CTL	Helper	VL
	40GAp	45CPp			
239	++	++	+++	++	R
204	-	+	+++	++	R
224	+	+	+	+++	NR
248	++	+	-	+	NR
214	+	++	+	++	NR
190	+	++	-	-	PR
31	++	++	++	++	NR
230	-	+	+	+++	R
234	++	++	+++	+	NR
138	++	++	+	+++	R

HIV-1-SPECIFIC IMMUNE RESPONSES

HU group (n=9)

OFF ART > w40

Patient	N Ab		CTL	Helper	VL
	40GAp	45CPp			
191	++	++	++	+	R
246	++	++	+	+++	R
236	++	++	+++	+++	R
143	++	++	+	++	PR
19	+	++	-	+	R
199	-	++	+	++	R
67	nt	nt	++	++++	R
241	+	++	-	-	NR
95	-	+	++	+	R

RESULTS

- After a median of 48 weeks of follow-up after STI, VL remained < 5,000 copies/ml (Responder patient) in 8/9 patients in the HU group and in 4/10 patients in the ART group ($p=0.02$).
- By STI#5, there was a significant increase in the HIV#45-specific NA from baseline ($p=0.003$) but no in the HIV#40-specific NA.
- An increase in both HIV-1-specific helper and CTL responses were observed at STI#5, without no differences between ART and HU groups at any time-point.
- There were no differences in the NA titers at any time-point comparing responders (R) and non-responders (NR) patients.
- There was a trend to higher HIV-1-specific helper and CTL responses in responder patients.

CONCLUSIONS

- **STI may induce effective CTL and helper responses in CHI patients associated with an spontaneous control of VL.**
- **A higher proportion of patients taking HU during on and off ART periods reached a VL set-point < 5,000 copies/ml.**
- **HU has no deleterious effects on HIV-1-specific neutralizing activity, helper or CTL responses.**
- **The effect on the control of viral replication could only be partially attributed to the HIV-1-specific immune responses.**
- **HU could be helpful as an immunomodulator in STI in chronic HIV-1-infected patients.**

ACKNOWLEDGMENTS

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