

# Effect of 24 weeks Intensification with a CCR5-antagonist on the Decay of the HIV-1 Latent Reservoir

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## Background

- Intensification of successful antiretroviral therapy has been proposed as a strategy that could control residual replication, diminish the HIV latent reservoirs, and so be useful for eradication purposes<sup>1</sup>.
- However, recently reported studies have failed to demonstrate any significant impact of treatment intensification on residual HIV-1 viremia in patients with chronic infection treated with HAART<sup>2,3</sup>
- As a consequence, intensification is closed to being discarded as useful for eradication purposes.

## Baseline characteristics of the patients

Patient	Gender	Age	CD4 count (cells/mm <sup>3</sup> )	CD8 count (cells/mm <sup>3</sup> )	HIV Viral load (copies/mL)	ART	Duration of ART (years)
1	M	49	1100	480	<50	zidVTC+VLP	12.4
2	M	47	486	673	<50	FTC+TDF+ATV	7.5
3	M	46	796	650	<50	3TC+ABC+ATV	7.8
4	F	46	787	1100	<50	FTC+TDF+LPV	5.7
5	M	45	534	764	<50	AZT+3TC+ABC	14.0
6	M	58	728	839	<50	AZT+3TC+ABC	14.4
7	M	32	694	1008	<50	FTC+TDF+ATV	5.0
8	M	30	1241	1414	<50	FTC+TDF+LPV	4.0
9	M	30	889	1064	<50	FTC+TDF+EV	3.8
Median (IQR)			717 (512-1072)	719 (525-950)	7.9 (4.0-14.4)		7.9 (3.8-14.4)

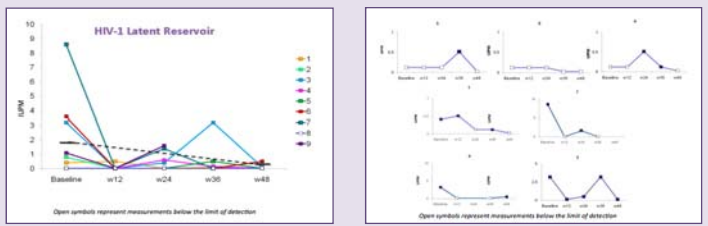
## Objetives

To evaluate the influence of treatment intensification with a CCR5 coreceptor antagonist (maraviroc, MVC) on: 1) HIV-1 latent reservoir; 2) Residual viremia; 3) Episomal DNA with 2 LTRs; 4) Immune markers.

The study, planned at 48 weeks, included patients with HIV chronic infection, with at least 3 ARV drugs, with VL < 50 copies/ml during at least 2 years, with CD4+ count above 350 cells/mm<sup>3</sup> and with an R5 tropism confirmed in stored plasma samples.



## HIV-1 Latent Reservoir in CD4 resting cells

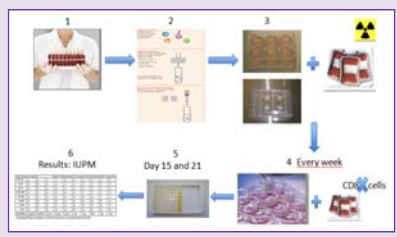


**Results 1:** After intensification with MVC, when compared to baseline, a decrease in the latent reservoir has been observed in most of the patients. Patient 2 and patient 9 discontinued early the study: one (patient 2) for toxicity and the other (patient 9) for poor adherence. Three patients showed values below the limit of detection at baseline and after 48 weeks. In the remaining 4 patients a decrease was observed compared to baseline, with undetectable values at week 48 in two of them.

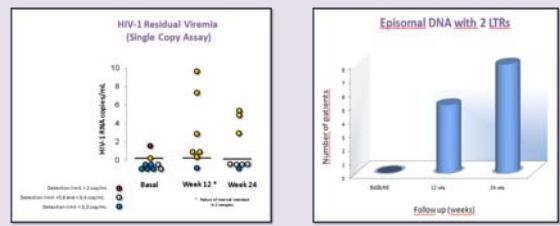
## Methods

### 1. Latent reservoir

A total of 450 mL of blood was extracted in lithium heparine tubes. The resting T CD4+ cells isolated and purified previously were plated in replicate dilutions and stimulated in a limiting dilution culture assay with allogenic irradiated PBMCs from a seronegative donor and PHA for 20 hours. Activated T resting CD4+ cells were co-cultured with PBMCs from a healthy donor after CD8+ T cell depletion (weekly). Replication competent HIV were quantified by HIV-1 p24 Antigen Assay in culture supernatants at days 5 and 21 (results are expressed in Infectious Units per Million, IUPM).



## Residual Viremia and Episomal DNA with 2 LTRs



**Results 2:** We observed an increase in the quantification of residual viremia and in the episomal DNA with 2-LTR after MVC intensification

### 2. Residual viremia

To quantify residual viremia below the threshold of detection of the techniques currently applied in clinical practice, we used the single copy assay described by Palmer S et al. (J Clin Microbiol 2003; 41: 4531-4536).

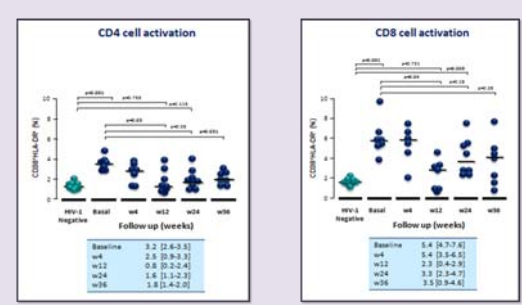
### 3. Qualitative 2-LTR-containing episomal circular DNA detection

Enriched episomal DNA was amplified by means of a nested PCR using primers flanking the 2-LTR junction designed by Sharkey et al. (J Virol 2005;79:5203-10).

### 4. Immune activation markers

We analysed the immune activation before MVC and after 12 and 24 weeks of MVC. The activation markers CD38 and HLA-DR (BD Bioscience) were used on CD4 and CD8 cells. The level of CD4 count and naïve CD4 count were also analysed.

## Immune Activation Markers



**Results 3:** A reduction in the proportion of activated CD4 and CD8 cells after MVC intensification was observed

## Conclusions

- After 48 weeks of intensification with MVC, a decrease in the size of the latent reservoir is observed in most patients evaluated. The quantification of the reduction is limited by the sensitivity of the method.
- In an apparent paradox, an increase in the residual viremia (SCA) and the episomal DNA with 2 LTRs is found in some of the patients evaluated
- A reduction in the proportion of activated CD4 and CD8 cells was observed.
- Overall, we feel that intensification with a CCR5 antagonist may have an impact on the reservoirs and deserves further evaluation

