Effect of Intensification with Raltegravir on HIV-1 Latently Infected CD4+ Memory T Cells

Carolina Gutiérrez1, Laura Díaz2, Alejandro Vallego1, Beatriz Hernández-Noya1, María Abad1, Nadia Madrid1, Fernando Dronda1, María Jesús Pérez-Elias1, Alba Moreno1, José Luis Casado1, Eduardo Muñoz1, María Ángeles Muñoz2 and Santiago Moreno1
1Hospital Universitario Ramón y Cajal, Madrid, Spain; 2Hospital General Universitario Gregorio Marañón, Madrid, Spain; 3Universidad de Córdoba. Servicio de Immunología, Córdoba, Spain

Introduction
Integrase mediates the integration of the HIV-1 DNA into the host genome. In the process of viral infection, inhibition of integration results in an irreversible block to HIV-1 replication. Most of the unintegrated viral DNA is degraded by a variety of cellular enzymes. Raltegravir (MK-0518, Merck) (RAL) constitutes the first available integrase strand transfer inhibitors (INSTIs) in clinical practice.

It has been suggested that the stability of the CD4 T cell reservoir could be related with the continuous replenishment from plasma residual HIV-1. Intensification with an INSTI like RAL could help eliminate the detectable levels of ongoing viral replication and accelerate the decay of the reservoir.

Objective:
To evaluate the influence of treatment intensification with RAL on: 1) HIV-1 latent reservoir; 2) Residual viremia; 3) Episomal DNA circles with 2 LTRs and 4) Immune activation markers.

Study Design
The study, planned to 48 weeks, included patients with HIV-1 chronic infection, with at least 3 HIV-1 RNA log copies/mL during at least 2 years, with CD4+ count above 350 cells/mm3.

Materials and methods

1-Latent reservoir
A total of 300 mL of blood was extracted in lithium heparine tubes. The resting CD4 T cells isolated and purified previously were plated in replicate dilutions and stimulated in a limiting dilution culture assay, with allogenic stimulated PBMCs from a seronegative donor and (PHA) for 20 hours. Activated resting CD4 T cells were co-cultured with PBMCs from a healthy donor and HIV-1 p24 antigenic peptide (Perkin Elmer) and Replication competent HIV were quantified by HIV-1 p24 Antigen Assay in cell culture supernatants at days 15 and 21 (results are expressed in Infectious Units per million).

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 (SCA) described by Palmer S et al. (J Clin Microbiol 2003; 41: 4531-4536).

3-Qualitative-3LTR-containing episomal circular DNA detection
Enriched episomal DNA was amplified by means of a real time PCR using primers from the 2LTR junction designed by Sharkey et al. (J Virol 2006; 80:5203-10).

4-Immune activation markers
We analyzed the immune activation before RAL and after 12, 24 and 36 weeks of RAL intensification. The activation markers CD38 and HLA-DR (BD Biosciences) were used on CD4 and CD8 T cells. The level of CD4 count and naïve CD4 count were also analyzed.

Results
Nine patients have been included. The median baseline CD4 count was 665 cells/mm3 (IQR 471-747). At 36 weeks we observed a decrease in the size of the latent reservoir (1.1 IUPM [0.2-4.4] BS vs. 0.0 IUPM [0.0-0.2] 36wks p=0.008) (Fig 1). No significant changes were observed in the episomal DNA circles: after 12 and 24 wks two patients became positive and only one patient became positive after 36wks intensification (Fig 2A). Using the SCA to quantify the residual viremia at 12wks an increase was observed in three patients compared with baseline determination (Fig 2B).

Conclusions
After 36 weeks of intensification with RAL we observed a significant decrease in the size of the latent reservoir.
- We observed an increase in the residual viremia, using the SCA in three patients although without global significance.
- We did not observe significant changes after RAL intensification in the presence of episomal DNA circles compare to baseline determination.
- No significant differences were found in the proportion of activated CD4 T cells. However the proportion of activated CD8 T cells tended to decrease.

Acknowledgements
We thank Carmen Pajo, Raquel Lizárraga, Eder Domínguez and Maria Corrales for laboratory assistance. We also thank Javier Zamora for his helpful in biostatistics analysis, and Belén Capuz and Diego Pedrero from Oncology Radiotherapy Department. We have a special acknowledgment to all participating patients and their families.

Founding Agencies:
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Table 1: Baseline Characteristics of the Patients

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Conferences
Presented at IAS 2011 – Rome, Italy

Figure 1: HIV-1 Latent Reservoir

Figure 2: Episomal DNA circles and SCA

Figure 3: Immune Activation Markers

No significant changes were observed in the episomal DNA circles after 12 and 24 wks two patients became positive and only one patient became positive after 36wks intensification (Fig 2A). Using the SCA to quantify the residual viremia at 12wk an increase was observed in three patients compare with baseline determination (Fig 2B).

A slight decrease in CD4 cell activation was observed but with no statistical significance. On the other hand, a decrease was observed in CD8 cell activation at 36wks, almost with statistical significance (3.82% [2.70-4.48] BS vs. 1.65% [1.35-3.48] 36wk p=0.069)

Conclusions
- After 36 weeks of intensification with RAL we observed a significant decrease in the size of the latent reservoir.

- We observed an increase in the residual viremia, using the SCA in three patients although without global significance.

- We did not observe significant changes after RAL intensification in the presence of episomal DNA circles compare to baseline determination.

- No significant differences were found in the proportion of activated CD4 T cells. However the proportion of activated CD8 T cells tended to decrease.

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