Immune activation throughout a boosted darunavir monotherapy simplification strategy

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Abstract

Our aim was to assess the evolution and the impact that blips, intermittent low-level viraemia and virological failure (VF) episodes have on patients' immune activation (IA) profiles during ritonavir-boosted darunavir monotherapy (mtDRV/rtv). A prospective cohort of human immunodeficiency virus-1-infected patients who switched to mtDRV/rtv was followed for 2 years. Cellular IA was assessed according to HLA-DR and CD38 expression in $CD4^+$ and $CD8^+$ T-cells and their naïve, effector memory and central memory subpopulations, and systemic IA was evaluated according to sCD14 and D-dimer levels. Seventy-five patients from the MonDAR cohort were selected for this substudy, and classified according to viral outcome as having continuous undetectable viraemia (n = 19), blips (n = 19), intermittent viraemia (n = 21), and VF (n = 16). The IA profile was closely linked to viral behaviour. Patients on viral suppression for 24 months showed a significant decrease in $CD4^+$ and $CD8^+$ T-cell activation and sCD14 and D-dimer levels. Patients with transient low-level viraemia episodes (blips and intermittent viraemia) showed cellular and systemic IA similar to baseline values. In contrast, significant increases in T-cell activation and sCD14 and D-dimer levels were observed in patients with VF. Baseline levels of $HLA\text{-}DR^+CD38^+CD8^+$ T-cells of >6.4% were independently associated with the emergence of VF. Therefore, mtDRV/rtv might be considered as a safe simplification strategy, on the basis of the IA results, whenever viral replication is under medium-term and long-term control. Transient low-level viraemia episodes do not affect patients' IA status. Moreover, $HLA\text{-}DR^+CD38^+CD8^+$ T-cell baseline levels should be considered when patients are switched to mtDRV/rtv.

Keywords: Boosted protease inhibitor monotherapy, HIV infection, immune activation
Original Submission: 23 September 2013; **Revised Submission:** 29 October 2013; **Accepted:** 4 November 2014
Editor: G. Antonelli
Article published online: 26 December 2013
Clin Microbiol Infect 2014; **20:** 1297–1303
10.1111/1469-0691.12521

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Introduction

Persistent immune activation (IA) is a hallmark of chronic human immunodeficiency virus (HIV) infection, even under long-term suppressive combined antiretroviral therapy. Higher levels of IA are associated with impaired $CD4^+$ T-cell reconstitution [1,2], higher mortality rates [3,4], and AIDS and non-AIDS-defining illnesses [5,6]. On the other hand, an increasing number of studies have demonstrated that ritonavir-boosted protease inhibitor monotherapy (mtPI/rtv), mainly based on lopinavir and darunavir, might be considered as an alternative strategy for treatment simplification in long-term virologically suppressed HIV-1-infected patients, with similar efficacy rates as triple therapy [7–17]. Nevertheless, important concerns regarding the safety of this therapeutic option remain, such as the increased incidence of blips and persistent viraemia episodes [18]. This residual viraemia might potentially induce a substantial increase in IA [19]; however, no studies of mtPI/rtv have addressed this issue to date. In this study, we assessed IA evolution throughout a period of darunavir/ritonavir monotherapy (mtDRV/rtv), and particularly the

influence that blip, intermittent viraemia and virological failure (VF) episodes have on the IA profile of HIV-1-infected patients.

Materials and Methods

Patients and study design

The MonDAR study (Clinical Trials.gov identifier: NCT01606722) prospectively enrolled all HIV-1-infected patients who were started on an mtDRV/rtv (800/100 mg once daily) simplification strategy at our outpatient clinic from January 2010 to April 2011 if they had: (i) a plasma HIV RNA level that was consistently <50 copies/mL for at least 6 months; and (ii) no resistance mutations that would confer decreased susceptibility to darunavir according to the International AIDS Society [20]. The study was conducted after informed consent had been obtained, according to the principles of the Declaration of Helsinki, and was approved by the Committee for Ethics in Biomedical Research of Andalucía and the Spanish Agency for Medicines.

Patients with available samples at months 0, 6, 12, 18 and 24 were included in this IA substudy, and were classified into four non-overlapping groups according to the following viral outcome on mtDRV/rtv based on a median of 10 viral load determinations per subject (interquartile range (IQR) 8–10): (i) continuous undetectable viraemia (CUV), for patients on viral suppression (<20 copies/mL) during the 24 months of the follow-up; (ii) blips, defined as transitory episodes of HIV RNA viral loads of >50 copies/mL, preceded and followed by a plasma viral load of <50 copies/mL without changes in the antiretroviral treatment; (iii) VF, defined as two consecutive viral load measurements of >200 copies/mL; and (iv) intermittent viraemia (IV), defined as episodes of detectable plasma HIV RNA during the follow-up without meeting the blip or VF criteria. Adherence was assessed by personal interview at each patient visit, and by analysis of the hospital pharmacy records.

Follow-up and laboratory procedures

Patient assessments were performed at baseline, after the first month on treatment, and every 3 months thereafter, including biochemical and haematological profiles, $CD4^+$ T-cell counts, and plasma HIV-1 viraemia (COBAS AmpliPrep/COBAS TaqMan HIV-1 Test, version 2.0; Roche Diagnostic Systems, Branchburg, NJ, USA), with a limit of detection of 20 copies/mL.

Blood samples were collected in Vacutainer cell preparation tubes (BD Biosciences, Madrid, Spain) at baseline and at each clinical visit during the 24 months of follow-up. We analysed samples from the CUV, IV and VF groups drawn at months 0, 6, 12, 18 and 24 of mtDRV/rtv treatment. Moreover, the IA profile changes in patients with blips and VF episodes were intensively analysed before each episode, at the moment of the viral rebound, and after each episode.

Peripheral blood mononuclear cell samples were stained with the following antibodies: αCD3-V450 (clone UCHT1), αCD4-APC-H7 (clone RPA-T4), αCD8-V500 (clone RPA-T8), αCD45RA-FITC (clone HI-100), αCD45RO-PE (clone UCHL1), αCCR7-PerCP-Cy5.5 (clone 150503), αCD38-APC (clone HIT-2), and αHLA-DR-PE-Cy7 (clone G46.6). Unstained controls and the following control antibodies were used: αCD4-APC-H7, αCD38-APC, αCD45RO-PE, and αHLA-DR-PE-Cy7. All of the antibodies were from BD Biosciences. Briefly, peripheral blood mononuclear cell samples were lysed with 100 μL of FACS lysing solution (BD Biosciences) to remove any remaining red blood cells. Then, the cells were washed with phosphate-buffered saline, stained for 30 min in the dark, washed again with phosphate-buffered saline, and resuspended in 1% paraformaldehyde. The cells were acquired with an LSR Fortessa flow cytometer (BD Biosciences), and at least 30 000 $CD3^+$ T-cells were analysed with FACSDiva software (BD Biosciences). IA was studied according to the single and double expression of HLA-DR and CD38 in T-cell subsets, grouped as naïve ($CD45RA^+CD45RO^-$), effector memory ($CD45RA^-CD45RO^+CCR7^+$) and central memory (T_{CM}; $CD45RA^-CD45RO^+CCR7^-$), as previously described [21,22].

Moreover, sCD14 levels (Human sCD14 Quantikine ELISA; R&D Systems, Abingdon, UK) and plasma D-dimer levels (Human D-Dimer ELISA Kit; CUSABIO, Wuhan, China) were quantified by the use of ELISA-based assays, following the manufacturer's recommendations, at baseline and after 24 months of mtDRV/rtv.

Statistical analysis

Results were expressed as median and IQR for continuous variables, and percentages for categorical variables. The Mann–Whitney *U*-test and Kruskal–Wallis *H*-test were used to compare the medians between the different groups, and the Wilcoxon and Freidman tests were used to compare patients within the same group. Correlations between age, time on highly active antiretroviral therapy or duration of viral suppression and IA markers (HLA-DR/CD38 expression in both $CD4^+$ and $CD8^+$ T-cells) were assessed with the Pearson correlation test. Receiver operating characteristic curves were constructed to discriminate between the different cut-off values of the IA markers at baseline, and their ability to predict VF on mtDRV/rtv, and areas under the curve of >0.65 were considered for further analysis. The cumulative incidence of VF during mtDRV/rtv treatment by month 24 as a function of the baseline levels of the IA markers was assessed with Kaplan–Meier