Immunovirological outcome and HIV-1 DNA decay in a small cohort of HIV-1-infected patients deintensificated from Abacavir/Lamivudine/Dolutegravir to Lamivudine plus Dolutegravir

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SUMMARY

Combination abacavir/lamivudine/dolutegravir (ABC/3TC/DTG) is approved as a first-line treatment for antiretroviral naïve patients. This report investigated the immunovirological outcome and total HIV-1 DNA decay in a small cohort of naïve HIV-1-positive patients treated with this regimen. In the presence of viral suppression and increased lymphocyte T CD4+ cells, the quantitative analysis of total HIV-1 DNA content revealed a significant decay after 12 months of treatment. Subsequently, we deintensificated the treatment of these patients from (ABC/3TC/DTG) to lamivudine plus dolutegravir (3TC/DTG) after 12 months of virological suppression, as a strategy of "induction-maintenance" therapy. The analysis of HIV-1 RNA viral load, total HIV-1 DNA, CD4+ T lymphocyte count and CD8+ HLA-DR+ T lymphocyte percentage after a mean 3.5 months of therapy deintensification showed no significant difference with respect to data detected after 12 months of ABC/3TC/DTG treatment in the presence of continuous viral suppression. These results indicate that the deintensification of highly active antiretroviral therapy (HAART) from ABC/3TC/DTG to 3TC/DTG effectively controls HIV-1 replication and in the early period does not induce any significant variations of total HIV-1 DNA. This suggests that HAART deintensification might be proposed as a therapeutic evolution in the treatment of HIV-1 infection.

INTRODUCTION

Dolutegravir (DTG) is the latest agent of the integrase strand transfer inhibitors (INSTIs) class approved for HIV infection treatment (Kandel and Walmsley, 2015). DTG and other components of the INSTI class (raltegravir and elvitegravir) act by preventing HIV-1 double strand DNA from incorporating in the host cell DNA, thus inhibiting HIV replication (Metiot *et al.*, 2013, Anstett *et al.*, 2017). DTG shows an increased potency, higher genetic barrier and minimal interaction with other drugs and is one of the best-tolerated antiretroviral drugs (Kanters *et al.*, 2016; Thierry *et al.*, 2017). DTG was co-formulated with abacavir and lamivudine and this combination (abacavir/lamivudine/dolutegravir; ABC/3TC/DTG) is now approved as a first-line treatment for use in HIV-1-infected patients (An-

tinori *et al.*, 2017). DTG performance in highly active antiretroviral therapy (HAART) was investigated in two randomized, double blind, non-inferiority trials (SPRING-2 and SINGLE; Raffi *et al.*, 2013; Walmsley *et al.*, 2013) and one open label, randomized phase III study (FLAMINGO; Molina *et al.*, 2015), in which DTG demonstrated its therapeutic impact. These results suggested the possibility to use antiretroviral-sparing strategies like dual therapies. "Less drug regimen" strategies could be used to avoid/prevent drug-related adverse events associated with the long-term use of HAART including the development of comorbidities such as renal, cardiovascular, liver diseases, or metabolic disorders (i.e. diabetes and dyslipidemia), more frequent in HIV-1-infected patients living longer (Borderi *et al.*, 2009; May *et al.*, 2014; Gallant *et al.*, 2017). The deintensification of therapy from a three drug regimen to a two drug treatment represents an interesting new avenue for antiretroviral therapy evolution (Marcotullio *et al.*, 2014; Amendola *et al.*, 2017). Among the different regimens for dual therapy, DTG associated with 3TC as "maintenance" treatment is an appealing possibility for therapy deintensification in patients with HIV-1 suppression (Boswell *et al.*, 2018) and this specific dual therapy is now under investigation. The present study reports the immunovirolog-

ical outcome and total HIV-1 DNA decay in a cohort of 20 HIV-1-infected patients treated with ABC/3TC/DTG, who were deintensificated to 3TC/DTG as an "induction-maintenance antiretroviral therapy" strategy after 12 months of viral suppression.

MATERIALS AND METHODS

From March 2016 to December 2016, the study enrolled 20 HIV-1-infected patients who started with ABC/3TC/DTG treatment. As indicated in the Italian National Guidelines (HIV/AIDS Italian Expert Panel 2016; Antinori *et al.,* 2016), we performed a genotypic resistance test (ViroSeq HIV-1 Genotyping System, Abbott, Chicago, IL, USA) for reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors (PIs) in all patients. No resistance mutations for NRTIs, NNRTIs, and PIs were shown. To determine viral genome mutations correlated to integrase inhibitors (INSTIs) resistance, ViroSeq HIV-1 Integrase Genotyping kit (Abbott) was employed (Wallis *et al.,* 2017). No HIV-1 seropositive patient was also co-infected with hepatitis B and/or hepatitis C viruses. All the enrolled subjects gave informed consent to all procedures in accordance with the Helsinki Declaration. Blood samples were collected at HIV-1 infection diagnosis before the onset of ABC/3TC/DTG therapy (T0) and during the follow-up at 3 (T3), 6 (T6), 9 (T9) and 12 (T12) months, and after therapy deintensification to 3TC/DTG (DITT; deintensification treatment time) for the quantitative determination of plasma HIV-1 RNA load. Total HIV-1 DNA content in peripheral blood mononuclear cells (PBMCs), CD4+ T lymphocytes, CD8+ T lymphocytes, and CD8+HLA-DR+ T lymphocytes were determined at T0, T12 and DITT. HIV-1 RNA load was determined using the COBAS AmpliPrep/COBAS TaqMan HIV-1 test (Roche Molecular Diagnostics, Basel, Switzerland) following the manufacturer's procedures. To determine total HIV-1 DNA load, whole blood samples were collected from HIV-1 seropositive patients by venepuncture in EDTA-containing tubes. PBMCs were separated by Ficoll gradient (Ficoll-Hystopaque d=1.077; Pharmacia, Uppsala, Sweden) following the manufacturer's indications. Cell pellets corresponding to $5x10^6$ cells were prepared and stored at -80°C until use. DNA from isolated PBMCs was extracted and purified with a QIAamp blood kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. The quantitation of total HIV-1 DNA load was carried out with a quantitative real-time PCR using primers and the amplification procedure described by Malnati and colleagues (Malnati *et al.,* 2008). Amplification and data acquisition were performed in Applied Biosystems Fast Dx real time PCR instrument (Applied Biosystems, Foster City, CA, USA). All standard dilutions, controls and samples were run in duplicate and the average value of the copy number was employed to quantify total HIV-1 DNA copies in PBMCs. Total HIV-1 DNA amount was normalized with the quantification of human *CCR-5* gene, as previously described (Malnati *et al.,* 2008). Total HIV-1 DNA data were expressed as number of copies/10^6 PBMCs. Exact values were used for calculations, ruling out decimal values. Statistical analyses were performed with Graph Pad Prism version 7 and Excel programs. The statistical methods employed in this study were: Mann-Whitney test, Wilcoxon test, Spearman test and Kruskall-Wallis test. The data were considered significant with p value <0.05.

RESULTS

This study enrolled 20 HIV-1 infected naïve patients (12 men and 8 women) who started ABC/3TC/DTG treatment from March 2016 to December 2016. Mean age of patients was 37.8 years (range 19-66 years). Four patients had AIDS-defining conditions at the beginning of highly active antiretroviral therapy (HAART). Mean and median HIV-1 RNA amount at baseline (T0) was 387.501 copies/mL (range 1760-6.230.000 copies/mL) and 75.700 (IQR 11168-100125), respectively.

Ten patients achieved viral suppression (HIV-1 RNA <20 copies/mL) at 1 month (all these patients had a HIV-1 RNA viral load <100.000 copies/mL at T0), 7 patients at 3 months (4 had a starting HIV-1 RNA viral load <100.000 copies/mL) and 2 patients (one with an HIV-1 RNA viral load <100.000 copies/mL) at 6 months. At February 2018, 17 patients were still on follow-up with a whole mean follow-up time of 18.4 months (range 14-22 months). Two patients exhibited virological failure and one was lost to follow-up after 3 months. The first patient failed the therapy after 6 months, with a viral load of 269 copies/mL (this patient was suppressed at 3 months) whereas the second patient displayed a virological failure after 14 months of therapy and 11 months of viral suppression with a viral load of 346 copies/mL. No resistance mutations for NRTIs or INSTIs were detected in the two failed patients who were switched to ABC/3TC and darunavir/cobicistat with rapid viral resuppression.

Among the remaining 17 patients, 4 displayed an HIV-1 RNA viral load value <20 copies/mL whereas 13 patients showed undetectable HIV-1 RNA content at T12. As expected, the decrease of HIV-1 RNA viral load was already significant when HIV-1 RNA viral load calculated in the plasma specimens of patients (n=17) at T0 were compared with those at T1 (p<0.0001; Wilcoxon matched pairs signed rank test; *Figure 1A*). We also evaluated the total HIV-1 DNA content and a significant direct correlation between HIV-1 RNA viral load and total HIV-1 DNA burden at T0 (n=17; p=0.04, R=0.50031; Spearman test) was observed. Total HIV-1 DNA (*Figure 1B*), significantly decreased between T0 (n=17; median 1040 copies/10^6 PBMCs; IQR 881-1156) and T12 (n=10; median value 376 copies/10^6 PBMCs; IQR 318-717; p<0.005; Mann-Whitney test).

The analysis of median CD4+ T lymphocyte count rose from 317 cells/µL (IQR 76-593) at T0 to 563 cells/µL at T12 (n=17; IQR 320-787; p<0.0001; Wilcoxon matched pairs signed rank test; *Figure 1C*). Median CD8+ T lymphocyte count decreased from 1154 cells/µL (n=17; IQR 751-1411) at T0 to 893 cells/µL at T12 (n=17; IQR 705-1231) and median CD8+ HLA-DR+ T lymphocyte percentage decreased from 35.5% at T0 (n=17; IQR 21.35-43.9) to 20.4% at T12 (n=17; IQR 11.7-24.3; p<0.001; Wilcoxon matched pairs signed rank test; *Figure 1D*).

The deintensification process to 3TC/DTG was implemented for 14 out of 17 patients with a mean follow-up time of 3.5 months (range 1-8 months; DITT). The HIV-1 RNA viral load of these 14 patients at last control after deintensification showed an HIV-1 RNA load <20 copies/mL in 3 patients and undetectable HIV-1 RNA load in 11, thus demonstrating that the dual therapy did not affect HIV-1 RNA viral load in the early period of dual therapy (p=0.88; Mann-Whitney test; *Figure 2A*). In parallel, we assessed total HIV-1 DNA (*Figure 2B*) in the same group of patients
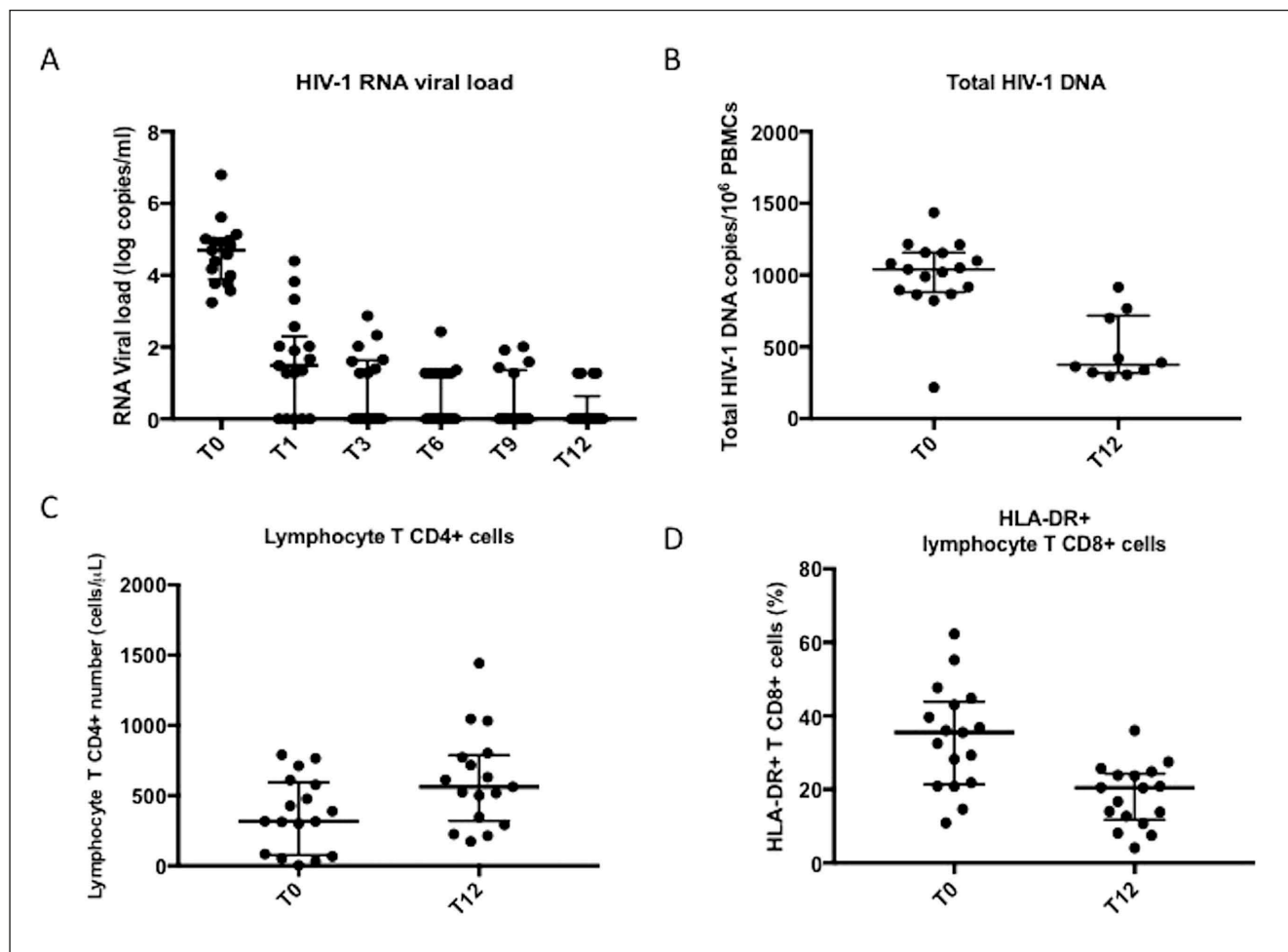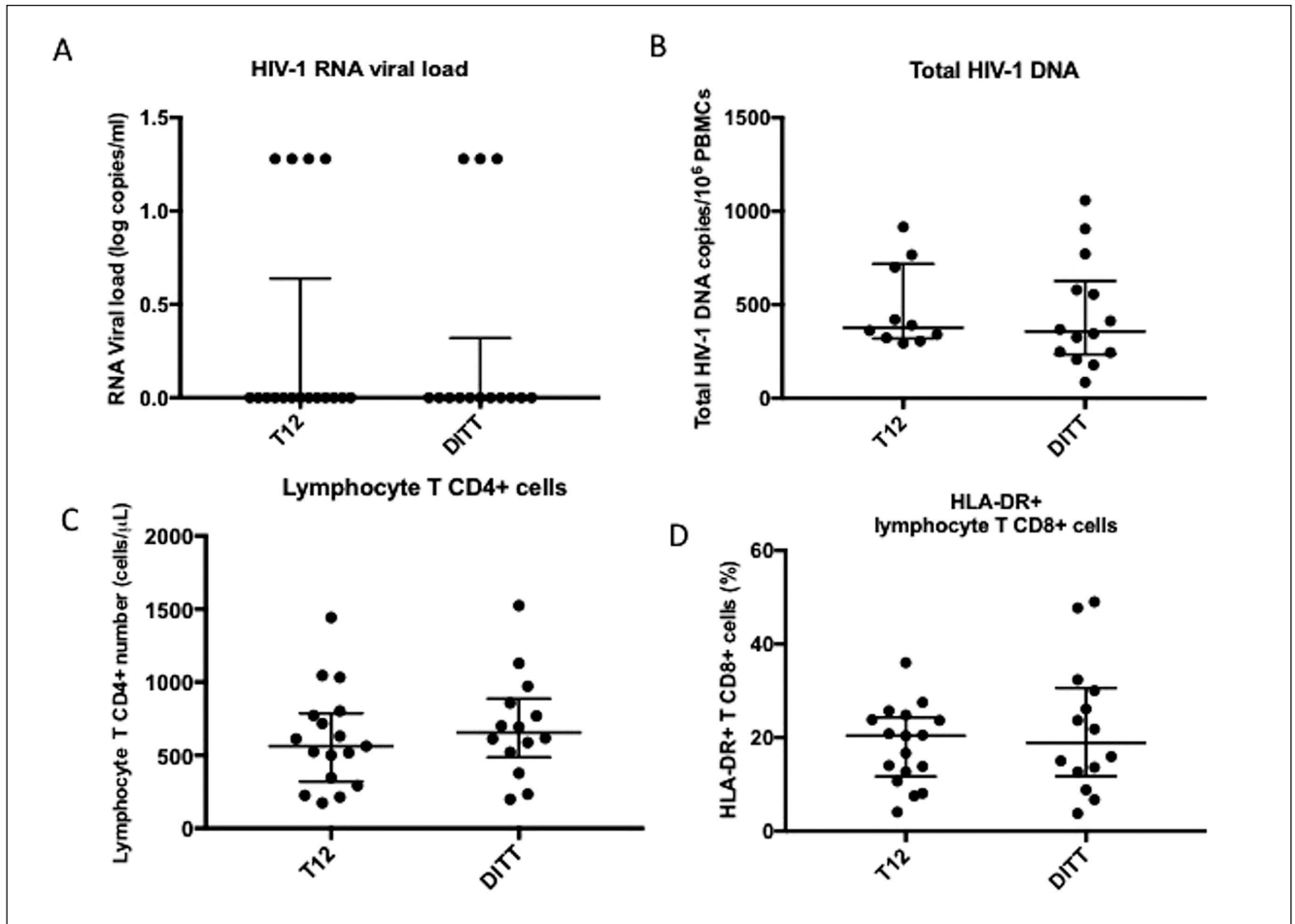
Figure 1 - *Virological and immunological parameters in the cohort of HIV-1 positive patients (n=17) at baseline (T0) and 12 (T12) months of treatment with ABC/3TC/DTG. In all panels, medians with interquartile range (IQR) are shown.* **Panel A**: *HIV-1 RNA viral load was expressed in logarithmic scale (Y axis). The plasma samples with HIV-1 RNA viral load <20 copies/mL were considered 19 copies/mL whereas samples with undetectable HIV-1 RNA load were considered 1. This panel also shows data at 1 (T1), 3 (T3), 6 (T6) and 9 (T9) months of treatment. The decay of HIV-1 RNA viral load was already significant when T0 and T1 were compared (p<0.001; Wilcoxon test).* **Panel B:** *Total HIV-1 DNA content in PBMCs achieved at baseline (T0; n=17), and 12 (T12; n=10) months of treatment. Total HIV-1 DNA decay was significant when T0 and T12 were compared (p<0.005; Mann-Whitney test).* **Panel C:** *Analysis of CD4+ T lymphocyte count at T0 and T12. The increase in CD4+ T lymphocytes is significant (p<0.0001; Wilcoxon test).* **Panel D**: *Analysis of CD8+ HLA DR+ T lymphocyte percentage at T0 and T12. The decrease of CD8+ HLA DR+ T cell percentage is significant (p<0.001; Wilcoxon test).*

treated with dual therapy (n=14; median value 356 copies/10^6 PBMCs; IQR 234-627**)**, demonstrating that the total HIV-1 DNA amount did not show significant variations at DITT (p=0.58; Mann-Whitney test) with respect to T12 samples (n=10; median value 376 copies/10^6 PBMCs; IQR 318-717). It is noteworthy that the decrease of total HIV-1 DNA was confirmed when the total HIV-1 DNA content at DITT was compared with T0 samples (n=17; p<0.001; Mann-Whitney test). The statistical comparative analysis of all three groups (T0, T12 and DITT) performed by Kruskal-Wallis test showed a significant p-value (p=0.0001).

The data analysis of CD4+ T lymphocytes, CD8+ T lymphocytes, and CD8+ HLA-DR+ T lymphocytes in the patients at DITT demonstrated a lack of significant variations when compared to data at T12 (*Figure 2, Table 1*). In these patients, CD4+ T cell count did not show significant variations between DITT (n=14; median value 656 cells/µL; IQR 485-886) and T12 samples (n=17; median value 563 cells/µL; IQR 320-787; p=0.42; Mann-Whitney test). Similarly,

CD8+ T lymphocytes calculated in the T12 group (n=17; median value 893 cells/µL; IQR 705-1231) and DITT group (n=14; median value 884 cells/µL; IQR 686-1248) did not display a significant p-value (p=0.8; Mann-Whitney test). CD8+ HLA-DR+ T cells showed a median percentage of 20.4% (IQR 11.7-24.3) and 18.85% (IQR 11.73-30.6; p=0.64; Mann-Whitney test) at T12 and DITT, respectively.

DISCUSSION

This report investigated immunological and virological parameters in a small cohort of HIV-1-infected naïve patients treated with ABC/3TC/DTG combination and, subsequently, deintensificated to 3TC/DTG dual therapy. Our pilot study confirmed the efficacy and tolerability profile of ABC/3TC/DTG treatment. It is noteworthy that no adverse events were observed in any subject. HIV-1 RNA viral load decay evaluation detected in 17 patients confirmed the consistent effect of DTG in rapidly reducing viral load in

Figure 2 - *Virological and immunological parameters in the cohort of HIV-1 positive patients treated with ABC/3TC/DTG at T12 (n=17) and deintensificated with 3TC/DTG at DITT (n=14). In all panels, medians with IQR are shown. **Panel A**: HIV-1 RNA viral load was expressed in logarithmic scale (Y axis). The plasma samples with HIV-1 RNA viral load <20 copies/mL were considered 19 copies/mL whereas samples with undetectable HIV-1 RNA load were considered 1. The decay of RNA viral load was not significant when T12 and DITT data were compared (p=0.88; Mann-Whitney test). **Panel B**: Total HIV-1 DNA content in PBMCs. Total HIV-1 DNA decay was not significant when T12 (n=10) and DITT (n=14) were compared (p=0.58; Mann-Whitney test). **Panel C**: Analysis of CD4+ T lymphocyte count at T12 (n=17) and DITT (n=14). The increase in CD4+ T lymphocyte count was not significant (p=0.8; Mann-Whitney test). **Panel D**: Analysis of CD8+ HLA DR+ T cell percentage at T12 (n=17) and DITT (n=14). The decrease of CD8+ HLA DR+ T lymphocyte percentage was not significant (p=0.64; Mann-Whitney test).*

Table 1 - *Lymphocyte subsets in HIV-1 infected patients.*

Lymphocyte subsets	T0 (cells/µL) n=17	%	T12 (cells/µL) n=17	%	DITT (cells/µL) n=14	%
CD4+ T lymphocytes (mean ±SD)	366.7±262.5	17.36±9.9	612.6± 338.3	27.17±10.45	699.7 ±350.6	31.21 ±9.16
CD4+ T lymphocytes (median)	317 (IQR 76-593)	18.5 (IQR 6.25-25.8)	563 (IQR 320-787)	31.4 (IQR 16.15-34.9)	656 (IQR 485-886)	33.4 (IQR 28.3-36.8)
CD8+ T lymphocytes (mean ±SD)	1120±613	56.68±14.78	1009±440.7	45.76 ±12.76	939.7±397.8	42.6±11.19
CD8+ T lymphocytes (median)	1154 (IQR 751-1411)	56.4 (IQR 43.95-69.55)	893 (IQR 705-1231)	44 (IQR 33.8-55.65)	884 (IQR 686-1248)	42.5 (IQR 32.68-50.78)
CD8+ HLA-DR+ T lymphocytes (mean ±SD)	-	34.11±14.02	-	18.28±8.37	-	21.94±14.04
CD8+ HLA-DR+ T lymphocytes (median)	-	35.5 (IQR 21.35-43.9)	-	20.4 (IQR 11.7-24.3)	-	18.85 (IQR 11.73-30.6)

T0: Baseline time; T12: 12 months of treatment with ABC/3TC/DTG; DITT: deintensification treatment time

plasma samples (Min *et al.*, 2011) and the data reported in several papers on ABC/3TC/DTG efficacy and safety (Maggiolo *et al.*, 2017; Comi *et al.*, 2018; Taiwo *et al.*, 2018). ABC/3TC/DTG therapy did not elicit HIV-1 suppression in two patients, probably due to a lack of adherence to treatment. In addition, ABC/3TC/DTG antiretroviral therapy was associated with an increase in CD4+ cells count and a decrease of HLA-DR+ lymphocyte T CD8+ percentage, a marker of immunoactivation. ABC/3TC/DTG treatment of HIV-1 naïve patients induced a significant decrease of total HIV-1 DNA in PBMCs. This effect was observed when we compared the total HIV-1 DNA content at T0 with the total HIV-1 DNA amount at T12. The treatment of HIV-1 naïve patients with different antiretroviral drug combinations, determined a decrease of HIV-1 RNA load and a decline in total HIV-1 DNA amount in both adults and children (Hoen *et al.*, 2007; Chomont *et al.*, 2009; Hocqueloux *et al.*, 2013; Jain *et al.*, 2013; Boulle *et al.*, 2014; Williams *et al.*, 2014; Cheret *et al.*, 2015). Total intracellular HIV-1 DNA is constituted by integrated proviral DNA, episomal unintegrated 1-LTR and 2- LTR, and linear unintegrated HIV-1 DNA. In quiescent lymphocyte T CD4+ cells, the 1-LTR and 2-LTR represent less than 10% of unintegrated forms. Total HIV-1 DNA is a biomarker of viral reservoir size and correlates to HIV-1 disease progression. High HIV-1 DNA content is associated with either more rapid progression to AIDS in HIV-1 naïve individuals or HIV-1 viral load rebound after HAART interruption in HIV-1-infected patients (Yearly *et al.*, 2004, Goujard *et al.*, 2006, Williams *et al.*, 2014). Furthermore, baseline total HIV-1 DNA burden might be considered a predictive factor for virological response to treatment: patients with higher total HIV-1 DNA content display a higher risk of virological failure or virological blips when the switch towards simplified therapy was used (Avettand-Fènoël *et al.*, 2010; Lambert-Niclot *et al.*, 2011; Geretti *et al.*, 2013; Prazuck *et al.*, 2013, Avettand-Fènoël *et al.*, 2016). The use of raltegravir, an integrase inhibitor, in naïve HIV-1 patients on combined antiretroviral therapy confirmed the HAART-dependent decline of total HIV-1 DNA (Koelsch *et al.*, 2011; Stephan *et al.*, 2014), while the switching of HAART to raltegravir-based regimens elicited a decrease of total HIV-1 DNA levels (Rossetti *et al.*, 2007; Michelini *et al.*, 2016) even though another report did not substantiate these observations (Lam *et al.*, 2012). On the other hand, the intensification of HAART therapy with raltegravir in experienced patients was not accompanied by a significant reduction of total HIV-1 DNA load (Collier *et al.*, 2016).

Deintensification and less-drug regimen strategies are considered a valuable approach for the long-term treatment of HIV-1 suppressed patients through the reduction of antiretroviral drug number and daily dose. This pharmacological simplification may promote a tailored treatment, a better tolerability and toxicity, the preservation of some treatment options, and a decrease of costs. Several studies showed that some dual therapy or monotherapy might maintain viral suppression (Arribas *et al.*, 2013; Casado *et al.*, 2016; Boswell *et al.*, 2018). We assayed HIV-1 RNA viral load and the total HIV-1 DNA determining that after 1-8 months of deintensification to 3TC/DTG both HIV-1 RNA and total HIV-1 DNA load were not significantly affected by this therapy switch suggesting that this treatment simplification does not influence the HIV-1 reservoir in the early period in the continuous presence of viral suppression.

In conclusion, the data derived from our cohort of patients confirmed the safety and potency of the ABC/3TC/DTG combination and the feasibility to deintesify to 3TC/DTG. These results suggest that the proposed deintensification might be used without any significant influence on viral replication control and the HIV-1 DNA reservoir in PBMCs not affected during the early period of treatment switch. Our study, however, is based on a small cohort of patients and a future study on a larger number of HIV-1 positive individuals is essential. In this respect, the results of a randomized trial (TANGO study) that will enroll approximately 550 adults with HIV-1 infection will be obviously more relevant in the comprehension of specific deintensification therapy effects.

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