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SHORT COMMUNICATION

Reduction of Immune Activation and Partial Recovery of Staphylococcal Enterotoxin B-Induced Cytokine Production After Switching to an Integrase Strand Transfer Inhibitor-Containing Regimen: Results from an Observational Cohort Study

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Abstract

Background and Objective Despite integrase strand transfer inhibitor (INSTI)-containing regimens now being considered a preferred option for both initial therapy and switching strategies in virologically suppressed patients, their effects on lymphocyte phenotypes and functions in the course of effective combination antiretroviral therapy (cART) are still unclear. Thus, we investigated the effect of a 24-week elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate (EVG/c/FTC/TDF) regimen on the T cell compartment and HIV reservoirs in HIV-infected patients switching from a suppressive protease inhibitor-based regimen.

Methods Thirty HIV-positive patients receiving suppressive tenofovir disoproxil fumarate/emtricitabine (TDF + FTC) (for a median of 5 years) in association with either darunavir/ritonavir (DVR/r) (47%) or atazanavir/ritonavir (ATV/r) (53%) were followed up for 24 weeks after switching to EVG/c/FTC/TDF. At baseline (week 0 [W0]) and after 12 (W12) and 24 (W24) weeks we analyzed HLA-DR (human leukocyte antigen–DR isotype)/CD38/Ki67/CCR7 (C-C chemokine receptor type 7)/CD45RA/CD127/PD-1 (programmed cell death-1) on CD4/CD8, interferon (IFN)-γ/interleukin (IL)-2 after HIV/Staphylococcal enterotoxin B (SEB) exposure (flow cytometry); total, integrated, and unintegrated HIV-DNA; and residual low-level HIV viremia (quantitative polymerase chain reaction [qPCR]).

Results While EVG/c/FTC/TDF introduction resulted in a stable CD4+ and CD8+ count, residual low-level HIV-RNA viremia, and HIV reservoirs, we observed a significant reduction in both activated CD4+ ($p = 0.016$) and CD8+ ($p = 0.048$) T cells, coupled with an increase in IL-2 and IFN-γ release by CD4+ and CD8+ effector memory T cells, and a decrease in cytokine production by terminally differentiated CD8+ T cells following SEB exposure. Furthermore, the magnitude of the reduction of activated HLA-DR + CD38 + CD8+ T cells ($r = -0.63$, $p = 0.014$) inversely correlates with the amount of total HIV-DNA at W24.

Conclusions Our data show a favorable effect of EVG/c/FTC/TDF switch to preserve immune activation-driven damage to T cell homeostasis, restore the multifunctional properties of effector T cells, and possibly contain cell-associated HIV viral burden in already virologically suppressed patients.

Key Points

Elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate (EVG/c/FTC/TDF) switch significantly reduces residual immune activation, while maintaining HIV viral suppression.

EVG/c/FTC/TDF switch partially restores T cell antibacterial properties.

In the long-term treatment strategy of HIV infection, switching to EVG/c/FTC/TDF might reduce the frequency of non-infectious co-morbidities by lowering the burden of residual activation/inflammation.

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1 Introduction

Given the viral efficacy coupled with improved tolerability and greater reported patient satisfaction [1], integrase strand transfer inhibitor (INSTI)-containing regimens are now recognized as the preferred first-line and switching strategies [2, 3].

The full control over HIV replication obtained by combination antiretroviral therapy (cART) [4, 5] has largely been proven not sufficient to completely reverse HIV-driven immune abnormalities such as heightened immune activation/inflammation and impaired immune functionality [6–10]. Some authors have suggested that regimens including an integrase inhibitor may reduce inflammation more effectively than other antiretroviral agents [11–17]. In particular, the AIDS Clinical Trials Group (ACTG) study A5248 reported a tentative normalization of monocyte activation markers (HLA-DR [human leukocyte antigen–DR isotype], CD86) following the initiation of open-label raltegravir plus tenofovir disoproxil fumarate/emtricitabine (TDF + FTC) [11]. A randomized clinical trial of elvitegravir/cobicistat/tenofovir disoproxil fumarate/emtricitabine (EVG/c/FTC/TDF) versus efavirenz/tenofovir disoproxil fumarate/emtricitabine (EFV/TDF/FTC) showed that initiation of the INSTI-based regimen was associated with a greater decline in highly sensitive C-reactive protein (hsCRP), soluble CD14 (sCD14), and lipoprotein-associated phospholipase A_2 (Lp-PLA_2) [12]. In contrast, Kelesidis et al. [13] did not find a clear pattern of changes in immune activation (both monocyte and lymphocyte) and inflammation markers, according to initial protease inhibitor (PI)- or INSTI-based regimens, suggesting an incomplete reversal of inflammation and immune activation in the setting of effective treatment, irrespective of the antiviral regimen.

The diverse effects on inflammation/activation between INSTI and other antiretroviral therapy (ART) classes seem to be even more evident in ART switch studies. Indeed, the SPIRAL (Switching From PI to RALtegravir in HIV Stable Patients) study showed an amelioration in levels of hsCRP, interleukin (IL)-6, tumor necrosis factor (TNF)-α, and D-dimer in the switch arm [14]. Similarly, in the ANRS (French National Agency for AIDS Research) 138 trial, switch to raltegravir from an enfuvirtide-based regimen resulted in improvements of pro-inflammatory markers IL-6, hsCRP, and D-dimer [15]. Lastly, the switch to raltegravir has been demonstrated to significantly reduce sCD14 in virally suppressed overweight women [16]. More recently, Villanueva-Millán et al. [17] have demonstrated that raltegravir-containing regimens are associated with lower inflammation, microbial translocation, and minor loss of bacterial species in the gut microbiota, as compared to both PI- and non-nucleoside reverse-transcriptase inhibitor (NNRTI)-based regimens, suggesting an involvement of INSTI-based cART in gut health, possibly contributing to the lower inflammation [17].

Given that the residual pro-inflammatory milieu in the course of virally suppressive cART has been associated with the onset of HIV-related non-communicable co-morbidities [18, 19], deep comprehension of the impact of specific ARV drug classes/regimens in containing excessive immune activation/inflammation is of the utmost importance. We therefore investigated the impact of a 24-week elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate (EVG/c/FTC/TDF) regimen on T lymphocyte phenotype and function and HIV reservoirs in HIV-infected patients switching from a suppressive PI-based regimen.

2 Patients and Methods

2.1 Study Design

This was a monocentric observational cohort study. HIV-infected patients on suppressive cART were consecutively enrolled to switch to (EVG/c/FTC/TDF) regimen. All patients were followed for 24 weeks post-switch. No control group was enrolled.

2.2 Patients

We consecutively enrolled 30 HIV-infected patients at the Clinic of Infectious Diseases, ASST Santi Paolo e Carlo, Department of Health Sciences, University of Milan, Milan, Italy. Patients were on stable tenofovir disoproxil fumarate/emtricitabine (TDF+FTC) + PI/ritonavir (PI/r)-based cART for at least 36 months, with undetectable plasma HIV-RNA viral load (< 40 cp/mL) in at least three consecutive assessments. The inclusion criteria were as follows: (1) TDF + FTC + PI/r-based cART for at least 3 years; (2) any CD4 count; and (3) HIV-RNA < 40 cp/mL for at least 6 months. The exclusion criteria were as follows: (1) acute HIV infection; (2) patients enrolled in trials with drugs that might influence the immune response; (3) subjects who in the investigator's judgment are non-compliant; (4) pregnant or breastfeeding women; (5) any serious illness that might affect survival; (6) creatinine clearance < 50 mL/min; (7) patients with glomerular filtration rate < 70 mL/min; (8) ALT > 5 above the range of normality; (9) treatment with an HIV-1 immunotherapeutic vaccine; (10) allergic reactions to the drug itself and/or to one of the excipients; or (11) evidence of viral resistance based on the presence of any resistance-associated exclusionary PI, INSTI, nucleoside reverse-transcriptase inhibitor (NRTI), or NNRTI mutation.

All of the enrolled patients provided written informed consent according to the Ethical Committee of our institution (Comitato Etico, ASST Santi Paolo e Carlo, Milan,

Italy). The ethics committee specifically approved this study (protocol no. 4386 10 April 2015 and approval no. 238 2 April 2015). All subjects gave written informed consent in accordance with the Declaration of Helsinki.

Peripheral blood samples were collected for subsequent analyses at baseline (week 0 [W0]) and after 12 (W12) and 24 (W24) weeks of EVG/c/FTC/TDF (1 tablet once daily).

2.3 Immunophenotype Analysis

Lymphocyte surface phenotypes were evaluated by flow cytometry on fresh peripheral blood, using the following fluorochrome-labeled antibodies: CD4-PE-Cy7, CD8-PE-Cy5, CD38-PE, HLA-DR-FITC, CD45RA-FITC, CCR7-PE, 7AAD, and PD-1-PE (BD Biosciences, San Jose, CA, USA), and CD127-PE (Beckman Coulter, Pasadena, CA, USA). The following combinations were used: CD4/CD8/CD38/HLA-DR (T cell activation), CD4/CD8/Ki67/CD127 (T cell proliferation/maturation), CD4/CD8/CD45RA/CCR7 (T cell maturation), and CD4/CD8/PD-1 (programmed cell death-1) (T cell exhaustion). T cell subsets were defined as naïve CCR (C-C chemokine receptor)7+CD45RA+, central memory (CM) CCR7+CD45RA−, effector memory (EM) CCR7−CD45RA−, and terminally differentiated (TD) CCR7−CD45RA+ subsets.

Briefly, 1×10^6 peripheral blood mononuclear cells (PBMCs) were stained with the appropriate antibodies for 20 min at 4 °C in the dark, and then washed and acquired using FACSVerse™ cytometer (BD Biosciences).

2.4 Antigen Stimulation

Ficoll-isolated PBMCs (1×10^6) were incubated in the presence of a pool of gag-env peptides (5 µM) or Staphylococcal enterotoxin B (SEB: 2.5 µg/mL; Sigma-Aldrich, Saint Louis, MO, USA). Brefeldin A (10 µg/mL, Sigma-Aldrich) and the co-stimulatory antibody CD28 (1 µg/mL, BD Biosciences) were added and cells were incubated over night at 37 °C with 5% CO_2. Intracellular detection of IL-2 and interferon (IFN)-γ was assessed by flow cytometry. The following antibodies were used: CD4-APC, CD3-APC-H7, CD45RA-PerCP-Cy5.5, CCR7-PE-Cy7, IL-2-PE, IFN-γ-FITC (BD Biosciences) and Viobility™ 405/520 Fixable Dye (Miltenyi Biotec, Bergisch Gladbach, Germany). Cells were harvested and stained with surface antibodies (CD3/CD4/CD8/CCR7/CD45RA/L/D/IL-2/IFN-γ). After paraformaldehyde (PFA) fixation (1%, Sigma-Aldrich), cells were permeabilized with Saponin 0.2% (Sigma-Aldrich) and stained with IL-2 and IFN-γ for 30 min at Room Temperature (RT). The percentage of IL-2- and IFN-γ-producing T cells assessed in medium alone were subtracted from SEB or HIV stimulation in order to reduce any possible bias due to the experimental procedure.

2.5 Soluble CD14 Quantification

Plasma levels of sCD14 were measured by an enzyme-linked immunosorbent assay (ELISA; R&D Systems, Minneapolis, MN, USA), according to the manufacturer's instructions.

2.6 CD4 Isolation

CD4 T lymphocytes were purified from freshly isolated PBMCs by negative selection using immuno-magnetic beads, according to the manufacturer's instructions (StemCell Technologies, Vancouver, BC, Canada).

2.7 HIV-RNA Quantification

Plasma HIV-RNA was quantified using the Abbott RealTime HIV-1 assay, with a detection limit of 40 cp/mL, according to the manufacturer's instruction. HIV-RNA copy numbers between 1 and 40 (residual low-level HIV viremia) were extrapolated from the standard curve of the assay (Abbott Laboratories, Princeton, NJ, USA).

2.8 Total, Unintegrated, and Integrated HIV-DNA Quantification

Cellular DNA was isolated from 2×10^6 CD4 and total, unintegrated, and integrated HIV-DNA forms were analyzed by a SYBR® (Diatheva s.r.l., Cartoceto [PU], Italy) Green I real-time quantitative polymerase chain reaction (qPCR) method using specific primers in the LTR-GAG highly conserved region of HIV-1 genome, as described in Casabianca et al. [20]. All polymerase chain (PCR) reactions were carried out in a 7500 Real-Time PCR system (Life Tech, Thermo Fisher Scientific Inc., Carlsbad, CA, USA) in a final volume of 100 µL using the HIV-1 DNA qPCR kit (Diatheva s.r.l., Cartoceto [PU], Italy) and according to the SYBR® Green I real-time qPCR [20] assay testing 0.5 µg (at least in duplicate) and 1.0 µg of cellular DNA. The HIV-1 DNA copy number was estimated by interpolation of the experimentally determined threshold cycle (Ct) on the standard curve (generated from 10^4 to 10, and 2-copy numbers). Values < 2 copies were arbitrarily considered to be 1 for statistical analyses. Total/unintegrated/integrated HIV-DNA copy numbers were normalized to 10^4 CD4 cells.

2.9 Statistical Analysis

Continuous variables were expressed as median and interquartile range (IQR), whereas categorical variables were expressed as absolute numbers and percentages. The Friedman test with Dunn's multiple comparison tests was used for statistics: a line below the *p* value showing which groups are being compared and for which the results were significant

has been added in each graph. The correlations among variables were tested by simple regression analysis (Spearman rank correlation). p values < 0.05 were considered statistically significant. Data were analyzed with GraphPad Prism version 6.2 (GraphPad Software Inc., San Diego, CA, USA).

3 Results

3.1 Patient Population

Patients were predominantly males (21/30; 70%), with a median age of 44 years (IQR 38–51), median HIV-infection duration of 8 years (IQR 5–20), and median time of HIV-RNA suppression and cART duration of 5 years (IQR 4–8.5) and 6 years (IQR 4.5–9), respectively (Table 1). At baseline, all patients were receiving TDF + FTC (for a median of 5 years) in association with either darunavir/ritonavir (DVR/r) (14/30; 47%) or atazanavir/ritonavir (ATV/r) (16/30; 53%) (Table 1). No hepatitis C virus (HCV)/hepatitis B virus (HBV) co-infections were found (Table 1). One patient dropped out due to adverse events (dizziness).

3.2 Modification of T Cell Compartment Following 24 Weeks of Elvitegravir/Cobicistat/Emtricitabine/Tenofovir Disoproxil Fumarate (EVG/c/FTC/TDF)

After 24 weeks the switch to EVG/c/FTC/TDF resulted in stable CD4+ and CD8+ counts, HIV reservoirs, and lipid profile over time (Table 2).

We next evaluated the impact of 12 and 24 weeks of EVG/c/FTC/TDF on T cell activation, finding a significant reduction in activated CD38+CD8+ T cells ($p = 0.018$; Fig. 1a), as well as HLA-DR+CD4+ ($p = 0.006$; Fig. 1b). Similarly, when we defined T cell activation as the co-expression of HLA-DR and CD38 on T lymphocytes, we found a significant decrease (CD8+ : $p = 0.048$ (Fig. 1c); CD4+ : $p = 0.016$ (Fig. 1d)). In contrast, we failed to observe any modification in circulating sCD14, a marker of monocyte activation ($p = 0.630$; Fig. 1e).

Interestingly, the decrease in T cell activation was associated with circulating HIV reservoirs. Indeed, the amount of total HIV-DNA after 24 weeks of EVG/c/FTC/TDF was inversely correlated with the magnitude of the reduction in activated HLA-DR+CD38+CD8+ T cells ($r = -0.63$, $p = 0.014$; Fig. 1f), supporting the close link between immune activation and circulating HIV viral reservoir.

Finally, we investigated T cell maturation, finding no major differences in both the CD4+ and CD8+ compartments (Table 3), with the exception of a modest decrease in CM CD4+ T cells ($p = 0.03$; Table 3). Also, no major changes in the proportion of proliferating, exhausted, and CD127-expressing CD4+ and CD8+ T cells were observed (Table 3).

Similar results were obtained stratifying the patients according to pre-switch PI/r exposure. Indeed, we observed a significant reduction of CD38+CD8+ ($p < 0.0001$) in patients previously exposed to ATV/r after 24 weeks of EVG/c/FTC/TDF (Fig. 2a–d). Similarly, patients taking DVR/r before the switch displayed a significant reduction of both CD4 (HLA-DR: $p = 0.007$; HLA-DR+CD38+: $p = 0.030$) and CD8 (CD38: $p = 0.038$; HLA-DR+CD38+: $p = 0.001$) T cell activation (Fig. 2e–h), with no other

Table 1 Clinical, epidemiological and viro-immunological features of the study population ($n = 30$)

Patients' characteristics	Value
Sex (male)	21 (70)
Age (years)	44 (38–51)
Risk factors	
Homosexual/bisexual	12 (40)
Heterosexual	16 (53)
IDU	2 (6)
HCV/HBV co-infection (yes)	0 (0)
Concomitant medications (yes)[a]	6 (20)
Time since first HIV diagnosis (years)	8 (5–20)
AIDS diagnosis (yes)	8 (27)
CD4 T cell count/mm^3	
Nadir	224.5 (53–297)
At time of analysis	573 (378–739)
HIV-RNA (Log copies/mL)	
Before cART	5.17 (4.19–5.81)
At time of analysis	1.59 (1.59–159)
Low-level HIV viremia (copies/mL)	12 (10–22)
cART duration (years)	6 (4.5–9)
HIV-RNA suppression duration (years)	5 (4–8.5)
Type of cART pre-switching to EVG/c/FTC/TDF	
Backbone	
TDF + FTC	30 (100)
Third drugs	
DVR/r	14 (47)
ATV/r	16 (53)
N of cART regimen pre-switching to EVG/c/FTC/TDF	
1	16 (53)
2	8 (27)
≥ 3	6 (20)

Data are presented as median (IQR) or absolute numbers (%)

ATV/r atazanavir/ritonavir, *cART* combination antiretroviral therapy, *DVR/r* darunavir/ritonavir, *EVG/c/FTC/TDF* elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate, *FTC* emtricitabine, *HBV* hepatitis B virus, *HCV* hepatitis C virus, *IDU* intravenous drug users, *IQR* interquartile range, *TDF* tenofovir disoproxil fumarate

[a]Concomitant medications include statins (HMG-CoA reductase inhibitors), fibrates, antihypertensives, and anticoagulants

Table 2 Immune recovery, HIV reservoirs, and lipid profile following 12 and 24 weeks of elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate switch

Variable	Week 0	Week 12	Week 24	*p* value
CD4+ T cell count (cell/mm^3)	634 (362–748)	614 (455–674)	582 (460–774)	0.456
CD8+ T cell count (cell/mm^3)	746 (668–903)	856 (571–1008)	829 (581–1049)	0.940
CD4/CD8 ratio	0.77 (0.54–1.03)	0.71 (0.56–0.89)	0.76 (0.58–0.97)	0.970
Low-level HIV-RNA viremia (copies/mL)	12 (10–22)	19 (0–39)	27 (24–39)	0.081
Total HIV-DNA (copies/10^4 CD4)	2 (1–4)	2.5 (1–5)	1 (1–5)	0.101
Integrated HIV-DNA (copies/10^4 CD4)	2 (1–5)	2 (1–3)	1 (1–3)	0.149
Unintegrated HIV-DNA (copies/10^4 CD4)	1 (0–1)	1 (0–1)	1 (1–1)	0.042
Total cholesterol (mg/dL)	185 (158–194)	183 (163–207)	180 (160–207)	0.147
HDL cholesterol (mg/dL)	46 (34–55)	48 (41–54)	50 (40–59)	0.309
LDL cholesterol (mg/dL)	112 (95–126)	109 (90–139)	107 (90–134)	0.555
Triglycerides (mg/dL)	102 (79–168)	96 (71–141)	85 (72–182)	0.254

Data are presented as median (IQR). Statistical analyses: Friedman test with Dunn's multiple comparison test

HDL high-density lipoprotein, *IQR* interquartile range, *LDL* low-density lipoprotein

differences in the remaining immunological markers (data not shown).

3.3 T Cell Cytokine Secretion upon Staphylococcal Enterotoxin B and HIV Challenge Following 24 Weeks of EVG/c/FTC/TDF

Given the data on persisting impairment of T cell responses upon cART initiation [21–24], we decided to further investigate IL-2 and IFN-γ production by different memory CD4+ and CD8+ T cell subsets, following ex vivo stimulation by HIV and SEB, known proxies of broad microbial challenge.

Figure 3a shows the gating strategy for the identification of cytokine production upon viral or bacterial challenge by flow cytometry.

Interestingly, upon SEB stimulation, EVG/c/FTC/TDF resulted in a restoration of IL-2-secreting, IFN-γ-secreting, and multifunctional IL-2/IFN-γ-secreting EM CCR7–CD45RA–CD4+ T cells ($p=0.011$, $p=0.0001$, $p=0.0001$, respectively; Fig. 3b). Similarly, after EVG/c/FTC/TDF switch, we found a rise in IFN-γ-secreting ($p=0.0001$) and IL-2/IFN-γ-secreting ($p=0.0001$) EM CCR7–CD45RA–CD8+ T cells (Fig. 3b), coupled with a parallel decrease of IFN-γ- and IL-2/IFN-γ-secreting TD CCR7–CD45RA+CD8+ T cells ($p=0.003$ and $p=0.0003$, respectively; Fig. 3b). No differences in cytokine production were found in the remaining CD4+ and CD8+ memory subsets following SEB exposure (data not shown)(Fig. 3).

Conversely, 24 weeks of EVG/c/FTC/TDF resulted in a very limited effect on HIV-specific response. Indeed, the initial cytokine production was very feeble, and was not restored after EVG/c/FTC/TDF introduction, aside from a modest increase (median [IQR]) of IFN-γ-secreting TD

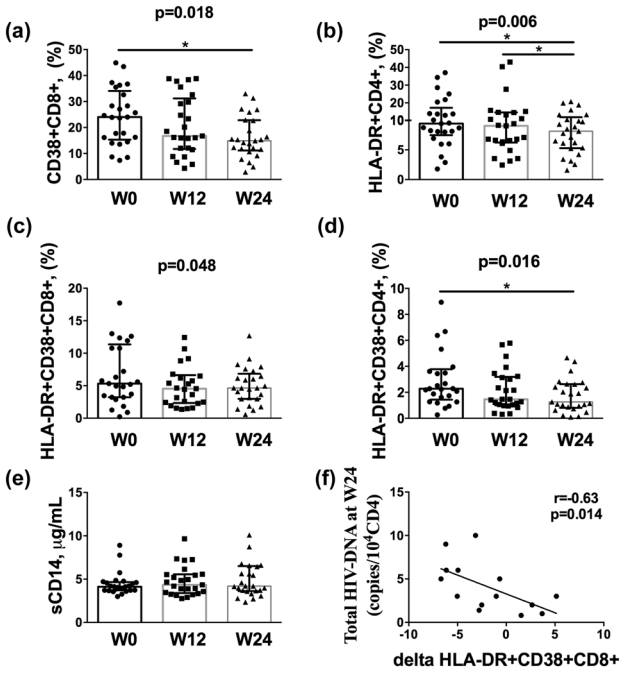


Fig. 1 T cell and monocyte activation following 24 weeks of EVG/c/FTC/TDF. In each graph the columns represent the median values, while the error bars indicate the interquartile range. Dots represent the patients' baseline values, squares represent the week 12 post-switch values, and triangles represent the week 24 post-switch values. The lines indicate a significant comparison between two groups. 12 and 24 weeks of EVG/c/FTC/TDF significantly reduced CD38+CD8+ T cells ($p=0.018$) (**a**), as well as HLA-DR+CD4+ ($p=0.006$) (**b**). We next defined T cell activation as the co-expression of HLA-DR and CD38 on T lymphocytes, finding a significant decrease in CD8+ ($p=0.048$) and CD4+ ($p=0.016$) (**c–d**). **e** We failed to observe any modification in circulating sCD14, a marker of monocyte activation ($p=0.630$). **f** Total HIV-DNA at week 24 was inversely correlated with the magnitude of the reduction of activated HLA-DR+CD38+CD8+ T cells ($r=-0.63$; $p=0.014$). *EVG/c/FTC/TDF* elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate, *HLA-DR* human leukocyte antigen–DR isotype, *sCD14* soluble CD14, *W* week, *$p<0.05$ for each pair of timepoints

CD4+ (W0: 0 [0–0] vs. W12: 0.21 [0–1.05] vs. W24: 0.04 [0–0.35]; $p = 0.038$) and IL2-secreting CM CD4+ T cells (W0: 0 [0–0.07] vs. W12: 0 [0–0.10] vs. W24: 0.27 [0.03–0.81]; $p = 0.0001$). No changes in cytokine production by the remaining CD4+ and CD8+ T cell subsets were observed upon HIV stimulation (data not shown).

An analogous behavior was observed according to pre-switch PI/r exposure (data not shown).

4 Discussion

Despite the reduced risk of death following cART introduction, HIV-positive subjects continue to have increased morbidity and mortality, as compared with the general population, often due to non-AIDS-related events [4, 25]. Persistently heightened systemic inflammation and immune activation that endure full viral suppression by cART have been described as key pathogenic players of non-AIDS comorbidities [10, 26].

Following their well-founded potency in gaining and maintaining optimal viral success [1], INSTI-based regimens are now widely recommended as both first-line and switching strategies. This in turn would raise the question on whether such potent regimens might also provide an added value in terms of immune activation/inflammation containment. Along this line, few data have been produced describing the immunological effects of INSTI-based regimens currently recommended as switching strategies in virally suppressed individuals [14–17, 27, 28] and the majority of these studies have focused on raltegravir. With this in mind, we decided to investigate the impact of 24 weeks of EVG/c/FTC/TDF on T cell compartment and HIV reservoirs in HIV-infected patients switched from a virally effective PI-based regimen.

In our cohort of HIV-infected patients with a long history of suppressive cART, 12 and 24 weeks of EVG/c/FTC/TDF resulted in substantial further reduction of T cell activation. These observations have important clinical implications given the involvement of immune activation in HIV disease progression [29]. The reasons behind this further decline in T lymphocyte activation burden in patients with long-term viral suppression are still unclear. One hypothesis is that the INSTI class may decrease inflammation and immune activation more than other antiretroviral classes because of their positive effects on lipid profiles, in particular on high-density lipoprotein cholesterol efflux capacity and oxidized low-density lipoprotein [30], known to be related to markers of monocyte activation that predict mortality [31]. This hypothesis, however, seems unlikely in our cohort because (1) we failed to find changes in lipid metabolism; and (2) we did not observe any modification in sCD14. In addition

Table 3 Immune phenotypes in a cohort of HIV-infected patients following 12 and 24 weeks of elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate switch

Phenotypes (%)	Week 0	Week 12	Week 24	*p* value
T cell maturation				
Naïve+CD4+	5.43 (2.3–7.28)	4.52 (1.49–6.49)	1.72 (0.53–6.92)	0.256
CM+CD4+	3.26 (2.25–6.40)	3.21 (2.09–5.08)	2.09 (0.32–6.65)	0.030
EM+CD4+	53.5 (44.2–67.7)	53.5 (41–63)	54.7 (45.2–61.1)	0.482
TD+CD4+	32.2 (15.4–40)	34.75 (24.4–44.4)	39.6 (24.3–49.2)	0.405
Naïve+CD8+	11.3 (2.9–22.3)	13.2 (3.03–21.4)	7.98 (4.54–18.9)	0.657
CM+CD8+	0.84 (0.31–3.01)	2.01 (0.64–4.41)	1.47 (0.54–2.64)	0.482
EM+CD8+	33.2 (22.3–43.8)	33.4 (25.6–47.2)	37.1 (27.5–43.5)	0.857
TD+CD8+	52.6 (34.7–60.3)	46.2 (29.4–61.0)	47.4 (35.3–58.6)	0.962
T cell proliferation				
Ki67+CD4+	2.12 (1.59–3.74)	2.44 (1.84–4.65)	3.34 (0.87–5.21)	0.846
Ki67+CD8+	1.83 (1.22–2.22)	1.91 (1.17–2.98)	1.81 (0.77–4.65)	0.747
T cell exhaustion				
PD-1+CD4+	3.84 (2.25–5.35)	2.84 (1.47–6.01)	2.79 (2.04–3.68)	0.289
PD-1+CD8+	4.15 (1.48–6.36)	3.16 (1.6–5.76)	3.29 (1.62–3.64)	0.339
T cell homeostasis				
CD127+CD4+	26.2 (20.5–35.9)	21.4 (10.2–33.1)	28.6 (20.1–34.5)	0.129
CD127+CD8+	18.6 (15.6–25.3)	21.8 (11.3–27.1)	23.3 (17.9–27.9)	0.072

Data are presented as median (IQR). Statistical analyses: Friedman test with Dunn's multiple comparison test

CCR7 C-C chemokine receptor type 7, *CM* central memory CCR7+CD45RA−, *EM* effector memory CCR7−CD45RA−, *IQR* interquartile range, *PD-1* programmed cell death-1, *TD* terminally differentiated CCR7−CD45RA+

to the metabolic benefits of INSTI, an alternative explanation for the decreased activation in these patients might be related to the better tissue penetration of these compounds [32]. Furthermore, integrase inhibitor-based regimens have been shown to more effectively reconstitute mucosal immunity, possibly reducing microbial translocation and the subsequent T cell immune activation [33].

Recently, an interesting in vitro model has been proposed, showing different kinetics of viral replication in macrophages and CD4 T cells according to the presence or absence of integrase inhibitors [34]. Several studies have described the effects of dolutegravir switching strategies on HIV reservoirs [28, 35], while treatment intensification studies have shown differential effects of raltegravir intensification on residual viremia and HIV reservoirs [36–40].

To date, aside from the study of Ngo Bell et al. [41], which demonstrated a high decay of blood HIV reservoirs with an elvitegravir-containing regimen in acute HIV infection, the understanding of the effects of elvitegravir-based switching strategies on HIV reservoirs is still lacking. In our cohort, we found that 24 weeks of EVG/c/FTC/TDF resulted in stable total, integrated, and unintegrated HIV-DNA forms and residual viremia, supporting the efficacy of this regimen in maintaining viral suppression. Interestingly, the inverse association between total HIV-DNA content and the magnitude of T cell activation reduction suggests that persistent immune activation in CD8+ T cells is driven, at least in part, by residual viral infection.

Aside from a higher risk of activation-mediated non-communicable diseases, HIV-infected individuals have a dramatic impairment of the antimicrobial defenses, exposing them to higher susceptibility to serious bacterial infections [42–45], and highlighting the need of antiretroviral strategies able to potentially improve T lymphocyte functions. To date, no data are available on the effects of EVG/c/FTC/TDF on T cell function. To fill this gap, we measured the production of IL-2 and IFN-γ by different memory CD4+ and CD8+ T cell subsets, following ex vivo SEB and HIV stimulation. Interestingly, while 24 weeks of EVG/c/FTC/TDF translated into a recovery of IL-2 and IFN-γ production by both CD4+ and CD8+ EM T cells following bacterial stimulation, no substantial

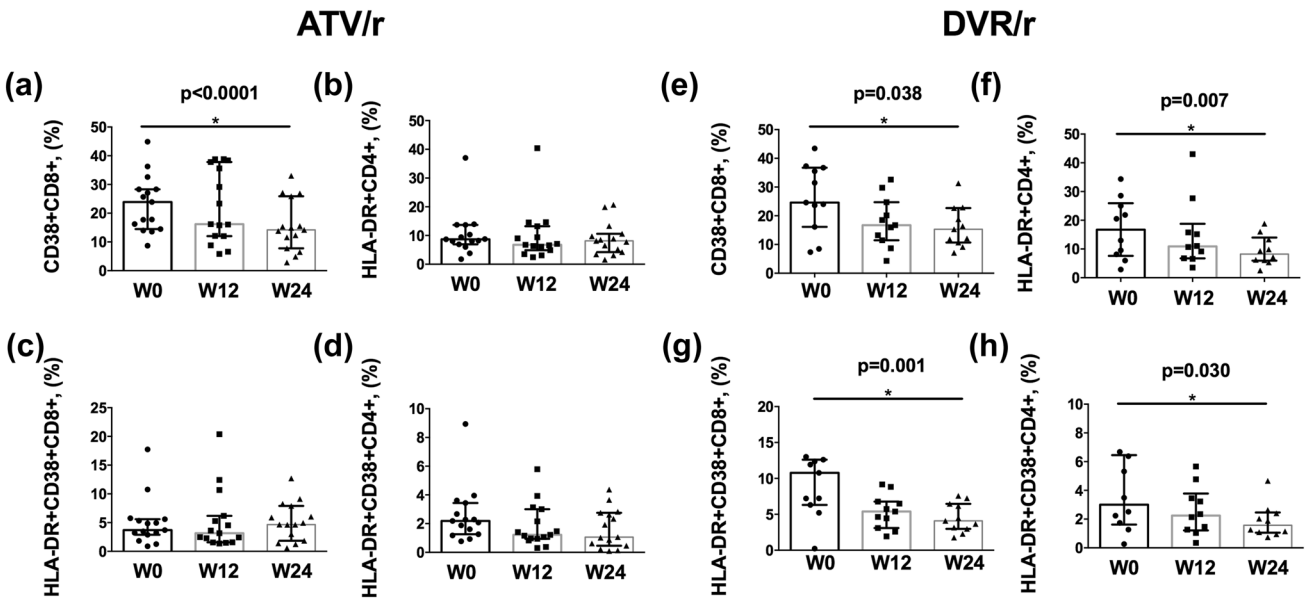
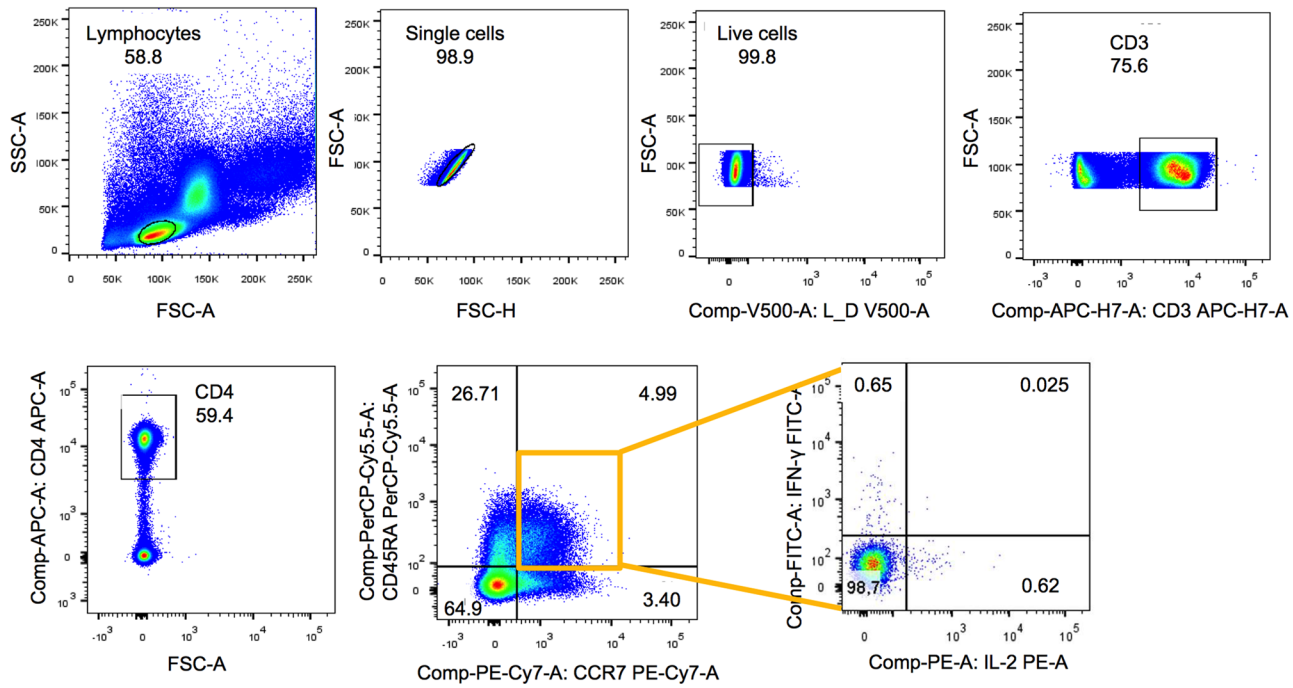
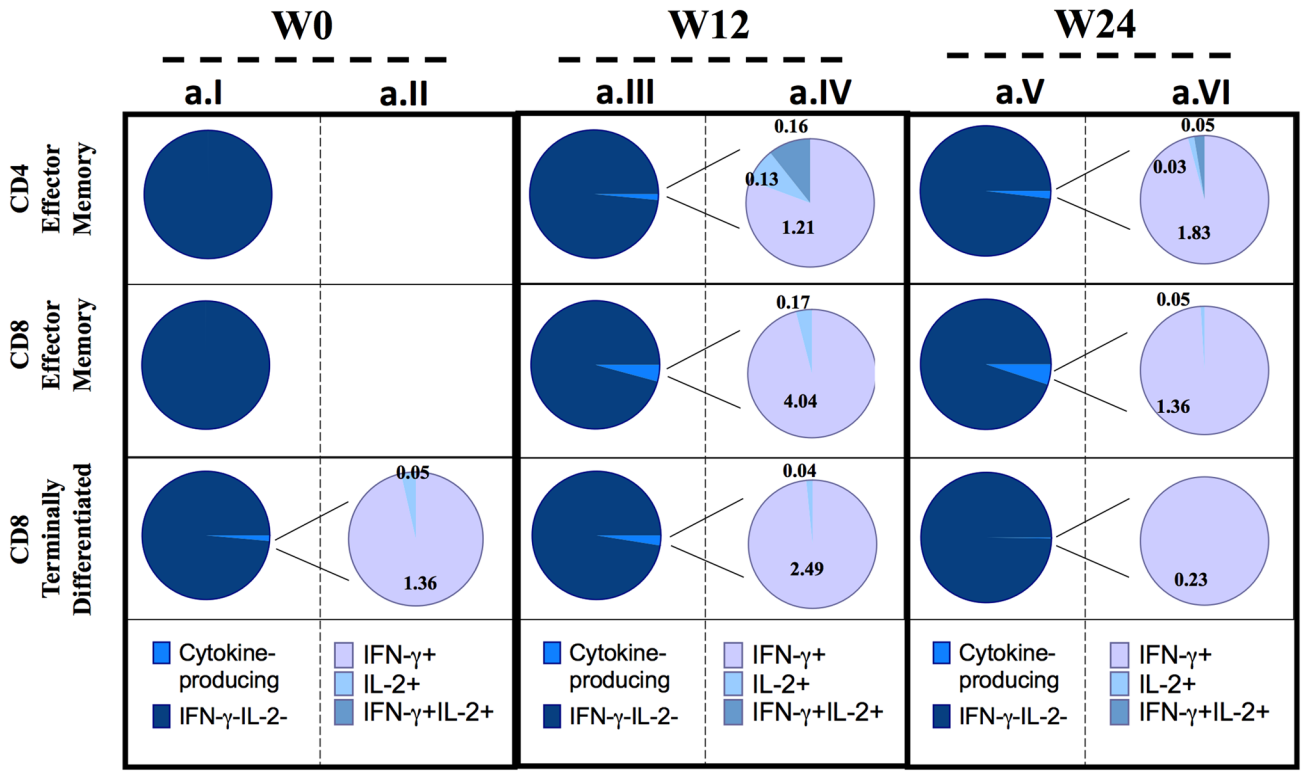


Fig. 2 T cell and monocyte activation following 24 weeks of EVG/c/FTC/TDF according to pre-switch PI/r-based regimen. In each graph the columns represent the median values, while the error bars indicate the interquartile range. Dots represent the patients baseline values, squares represent the week 12 post-switch values, and triangles represent the week 24 post-switch values. The lines indicate a significant comparison between two groups. **a–d** Modification of T cell activation in patients on TDF/FTC + ATV/r before the switch. **a** 12 and 24 weeks of EVG/c/FTC/TDF significantly reduced CD38 + CD8+ T cells ($p < 0.0001$). **b** No changes in HLA-DR + CD4+ T cells after 12 and 24 weeks of EVG/c/FTC/TDF. **c** Similar levels of HLA-DR+CD38+ expressing CD8 T cells at W12 and W24 post-EVG/c/FTC/TDF. **d** Not significant reduction in HLA-DR+CD38+CD4 T cells after 12 and 24 weeks of EVG/c/FTC/TDF. **e–h** Changes in T cell activation in patients receiving TDF/FTC+DVR/r before the switch. **e** 12 and 24 weeks of EVG/c/FTC/TDF significantly reduced CD38+CD8+ T cells ($p = 0.038$). **f** Significant reduction in HLA-DR+CD4+ T cells after 12 and 24 weeks of EVG/c/FTC/TDF ($p = 0.007$). **g** HLA-DR+CD38+ expressing CD8 T cells were significantly reduced at W12 and W24 post-EVG/c/FTC/TDF ($p = 0.001$). **h** Significant reduction in HLA-DR+CD38+CD4 T cells after 12 and 24 weeks of EVG/c/FTC/TDF ($p = 0.030$). *ATV/r* atazanavir/ritonavir, *DVR/r* darunavir/ritonavir, *EVG/c/FTC/TDF* elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate, *HLA-DR* human leukocyte antigen–DR isotype, *PI/r* protease inhibitor/ritonavir, *TDF/FTC* tenofovir disoproxil fumarate/emtricitabine, *W* week, $*p < 0.05$ for each pair of timepoints

(a) Gating Strategy



(b) SEB Stimulation



◂**Fig. 3** T cell cytokine secretion upon SEB challenge following 24 weeks of EVG/c/FTC/TDF. **a** Lymphocytes were gated from FSC and SSC, doublets were removed, and live cells were selected, segregated for CD3+ T cells and subsequently for CD4+ T cells (or CD8+). CD4+ T cell subsets were defined as naïve CCR7+CD45RA+, central memory CCR7+CD45RA−, effector memory CCR7−CD45RA−, and terminally differentiated CCR7−CD45RA+ subsets. Within each subset, IL-2 and/or IFN-γ release was assessed. The gates were set up based on positive versus negative peak. **b** SEB stimulation. a.I, a.III, and a.V show pie charts representing the cytokine production following bacterial stimulation by T cells (− indicates non-producing and + indicates producing cells). a.II, a.IV, and a.VI detail the percentage of cytokine-producing T cell subsets (− indicates non-producing and + indicates producing cells). At week 0, the proportion of cytokine-producing T cells was negligible. Interestingly, 24 weeks of EVG/c/FTC/TDF resulted in a partial restoration of IL-2-secreting, IFN-γ-secreting, and multifunctional IL-2/IFN-γ-secreting CD4+ and CD8+ effector memory, as well as CD8+ terminally differentiated T cells. *APC* allophycocyanin, *CCR* C-C chemokine receptor, *Comp* compensated, *EVG/c/FTC/TDF* elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate, *FSC* forward scatters, *FITC* fluorescein isothiocyanate *IFN-γ* interferon-γ, *IL-2* interleukin-2, *PerCP* peridinin chlorophyll protein complex, *PE* phycoerythrin, *SEB* staphylococcal enterotoxin B, *SSC* side scatter, *W* week

modifications in HIV-specific response were seen. The increase in IL-2 and IFN-γ release by EM T cells, coupled with a decrease in cytokine production by TD CD8+ T cells, may suggest a role of EVG/c/FTC/TDF in restoring the multifunctional properties of effector T cells. In contrast, the persistent impairment of HIV-specific response seems to imply profound damage to antiviral immune function, possibly early during the infection, that is not restored by suppressive cART.

Several limitations in the present study need to be acknowledged, such as the lack of a control group, limited sample size, and short follow-up period. Indeed, our findings should be corroborated in larger patient cohorts and with the appropriate controls.

5 Conclusion

Our data suggest a favorable effect of EVG/c/FTC/TDF on preserving immune activation-driven damage to T cell homeostasis, restoring the multifunctional properties of effector T cells, and possibly containing cell-associated HIV viral burden in virologically suppressed patients. As long as viral suppression remains the goal of HIV treatment, choosing the cART with the least long-term toxicity and highest benefit is a priority in the management of this chronic illness. However, viral persistence and residual inflammation are interdependent and fuel each other in a vicious cycle that seems difficult to interrupt [46]. For this reason, reducing activation/inflammation may be an efficient way to interfere with the maintenance of the HIV reservoir in virally suppressed individuals on ART.

The potential benefits of the EVG/c/FTC/TDF regimen, which maintained virologic efficacy and further decreased immune activation, warrant further investigation to uncover the association of this therapy with the potential decrease in the morbidity and mortality of HIV-infected patients due to non-AIDS-related illnesses.

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Compliance with Ethical Standards

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Conflict of interest EM., F.A.C., A.C., C.O., M.M., G.A., C.T., and A.D.M. declare that they have no conflict of interests. G.M. received Unrestricted Educational Grant no. IN-IT-236-1320 from Gilead Italia.

Ethics approval All procedures in this study were in accordance with the 1964 Helsinki Declaration (and its amendments). The ethics committee of ASST Santi Paolo e Carlo specifically approved this study (protocol no. 4386 10 April 2015 and approval no. 238 2 April 2015).

Informed consent All subjects gave written informed consent in accordance with the Declaration of Helsinki.

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