Effect of 12-month Switch to Dual cART on HIV reservoir, T-cell homeostasis, Gut and Mitochondrial Damage

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UNIVERSITÀ DEGLI STUDI DI MILANO

Sistema Socio Sanitario Regione Lombardia ASST Santi Paolo e Carlo

Valeria Bono[1], Camilla Tincati[1], Matteo Augello[1], Roberta Rovito[1], Sabrina Marozin[1], Chiara Orlandi[2], Anna Casabianca[2], Giulia Marchetti[1]

[1] *Clinic of Infectious Diseases, Department of Health Sciences, ASST Santi Paolo e Carlo, University of Milan, Milan;*

[2]*Department of Biomolecular Sciences, Section of Biochemistry and Biotechnology, University of Urbino Carlo Bo, Fano (PU)*

Contacts: valeria.bono@unimi.it, giulia.marchetti@unimi.it

BACKGROUND

Residual immune activation/inflammation is a hallmark of treated HIV. Dual (2DR) regimens are efficacious in maintaining viral suppression, however, their effect on HIV reservoir, T-cell homeostasis and mitochondrial damage, is ill-defined.

METHODS

60 PLWH switching from a virologically suppressive three-drug (3DR) cART regimen to an INSTI-based two-drug regimen (2DR), with sample availability at the time of switch and approximately 12 months post-switch, were studied (Table1). We assessed the HIV reservoir (total, unintegrated, and integrated HIV DNA) by qPCR, T-cell surface maturation (CD127/CD45RA) and activation (CD38/CD45RO) by flow cytometry, plasma markers of gut barrier dysfunction (E-cadherin, I-FABP) and microbial translocation (sCD14, LBP, ELISA; 16S rDNA, qPCR), as well as circulating cell-free mitochondrial DNA (ccf-mtDNA, qPCR). Statistical analysis was performed using the Wilcoxon test and Spearman's correlation test.

Table 1. Demographic and clinical features of the study population (n=60) on suppressive 3DR cART (pre-switch)

Age, years, median (IQR)	**48 (42-58)**
Sex, n (%)	
Male	**50 (83.33%)**
Female	**10 (16.66%)**
Ethnicity, n (%)	
Caucasian	**55 (91.66%)**
Asian	**2 (3.33%)**
Hispanic	**3 (5%)**
Risk factors for HIV infection, n (%)	
MSM	**40 (66.66%)**
Eterosexual	**17 (28.33%)**
IDU	**3 (5%)**
BMI, median (IQR)	**25 (23-26.7)**
Smokers, n (%)	**20 (33.33%)**
Alcohol use, n (%)	**47 (78.33%)**
Comorbidities	
History of cardiovascular diseases, n (%) (Dyslipidemia, HTA, Previous IMA, NAFLD ecc.)	**20 (33.33%)**
History of cancer, n(%)	**19 (31.66%)**
HBV (previous or vaccinated), n (%)	**29 (48.33%)**
Co-infection HCV, n (%)	**4 (6.66%)**
Time since HIV diagnosis (years), median (IQR)	**14 (10-18)**
Time from diagnosis to cART start (months), median (IQR)	**0 (0-4)**
Time on cART (years), median (IQR)	**13 (10-18)**
Time from switch (months), median (IQR)	**11 (8-14.7)**
CD4 cell count nadir (cells/mm3), median (IQR)	**275 (182.5-407.5)**
Current %CD4, median (IQR)	**32 (27-39)**
Current CD4, cells/ml, median (IQR)	**700 (543-937)**
Total time with viral load <50cps/mL (years), median (IQR)	**9 (7-9)**
Time since last viral load ≥50cps/mL (years), median (IQR)	**9 (7-9)**
Previous virological failure, n (%)	**1 (1.66%)**
Past AIDS-defining events (CDC C), n (%)	**9 (15%)**
Number of cART lines prior to switch, n (%)	
0-3	**47 (78.33%)**
4-8	**11 (18.33%)**
3DR cART prior to switch, n (%)	
INSTI-based	**41 (68.33%)**
NNRTI-based	**14 (23.33%)**
PI-based	**4 (6.66)**
INSTI + PI-based	**1 (1.66%)**
2DR cART regimen post switch, n (%)	
3TC/DTG	**53 (88.33%)**
DTG/RPV	**7 (11.66%)**

RESULTS

No significant changes in either total HIV DNA levels (p=0.3), unintegrated (p=0.3) and integrated (p=0.4) DNA levels were observed after the switch to 2DR (Fig 1A). Switching to 2DR led to significant CD4 increases (32% [27-39] vs 35% [30-40]), reduction in CD8 lymphocytes (40% [34-45] vs 37% [30-42]) and rise in CD4/CD8 ratio (0.83 [0.65- 0.91] vs 0.96 [0.76-1.3], Fig. 2A-C).

Switching to 2DR cART in virologically-suppressed PLWH had a differential effect on T-cell homeostasis, increasing central memory cells and decreasing activated cells, while also diminishing the naïve T-cell pool. In contrast, 2DR cART did not impact the viral reservoir, gut-driven microbial translocation, or cellular stress, all of which did not appear to be associated with the changes in T-cell phenotypes. Of note, our data also revealed a persistent correlation between circulating 16SrDNA and ccf-mtDNA, suggesting an effect of microbial products in driving cellular damage that seems not affected by cART regimen.

Figure 1.
Total, unintegrated and integrated HIV DNA

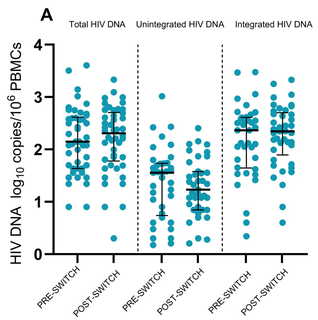


Figure 2.
CD4 and CD8 T-cell counts pre- and post-switch to 2DR

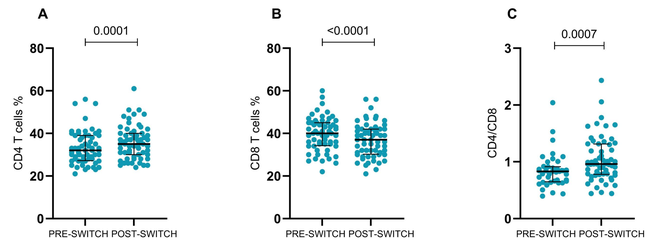


Figure 3.
CD4 and CD8 T-cell maturation pre- and post-switch to 2DR

Moreover, switch to 2DR resulted in a decrease of naïve T cells (CD4/CD45RA: 11% [8-17] vs 10% [6-13]; CD8/CD45RA: 18% [15-22] vs 17% [12-20], and significant expansion of central memory phenotypes (CD4/CD127: 28% [24-33] vs 32% [26-37]; CD8/CD127: 30% [24-34] vs 32% [26-36], Fig. 3A-D).

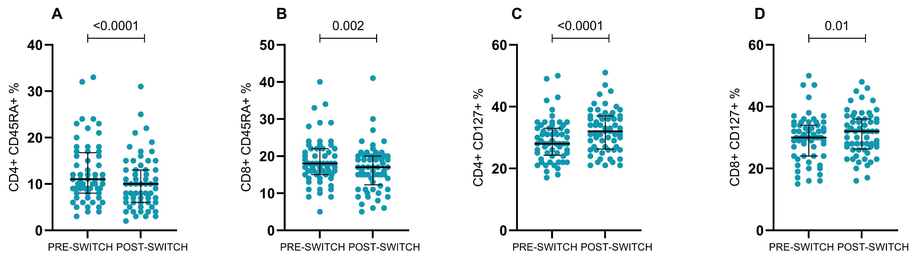


Figure 4.
CD8 T-cell activation pre- and post-switch to 2DR

A marked reduction in activation phenotypes was observed after switch to 2DR therapy (CD8/CD38: 5% [4-8] vs 3% [2-5]; CD8/CD45RO: 7% [4-11] vs 6% [3-8]; CD8/CD38/CD45RO: 1% [0-1] vs 0% [0-1], Fig. 4A-C).

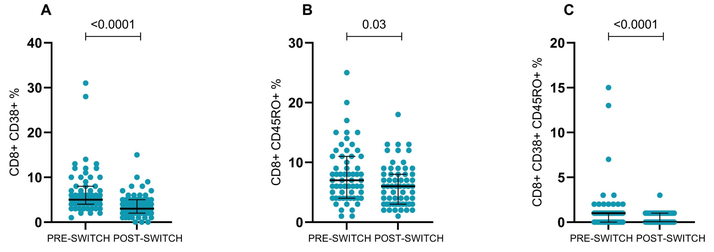


Figure 5.
Gut barrier damage, monocyte activation and microbial translocation pre- and post-switch to 2DR

No changes in markers of intestinal damage and microbial translocation were detected after the switch (Fig. 5A-E).

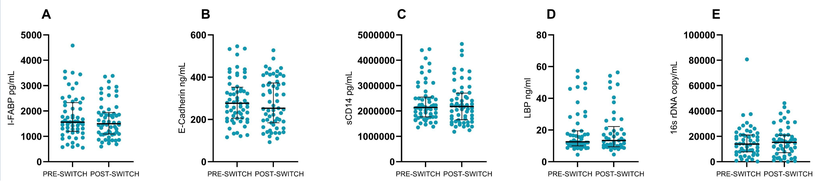


Figure 6.
Circulating cell-free mitochondrial DNA (ccf-mtDNA) pre- and post-switch to 2DR

Notably, ccf-mtDNA levels remained unchanged one year after the switch to 2DR, and this was consistent in all three genes analysed individually (Fig. 6A-D).

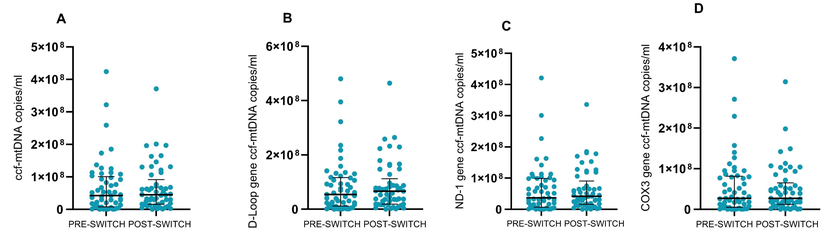
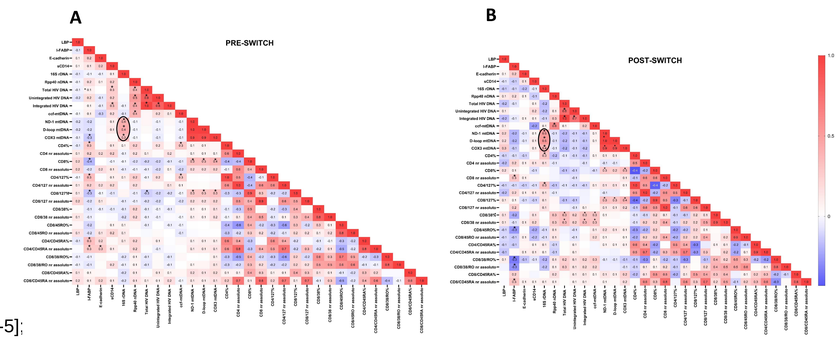


Figure 7.
Heatmap of correlations between HIV DNA, microbial translocation, T cell- homeostasis and mitochondrial damage markers.

No correlations were found between T-cell homeostasis, HIV reservoirs, gut damage/microbial translocation and mitochondrial stress (Figure 7). 16S rDNA and ccf-mtDNA were strictly linked both prior to (r=0.4, p=0.0002; Fig. 7A) and after the switch to 2DR (r=0.5, p=0.0002) (Fig. 7B).



CONCLUSIONS

cART switch from 3DR to 2DR had a differential effect on T-cell dynamics: on the one hand, it increased central memory cells and reduced CD8+ T-cell activation; on the other, it diminished naïve T-cell frequencies. These changes in T-cell homeostasis did not appear to be driven by modifications in the viral reservoir, gut damage/microbial translocation, or mitochondrial stress, which remained stable regardless of therapy modification. Of note, the correlation between 16S rDNA and ccf-mtDNA both prior and after switch to 2DR, suggests a persistent link between microbial translocation and cellular stress which is not affected by the change in cART regimen. Further research is required to uncover the underlying mechanisms driving these findings.