



## Original article

# Immunological and inflammatory changes after simplifying to dual therapy in virologically suppressed HIV-infected patients through week 96 in a randomized trial

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## ABSTRACT

**Objectives:** To evaluate whether simplification of antiretroviral treatment to dual therapy (DT) negatively impacts immune recovery (IR), immune activation and inflammation (IA/I), and HIV reservoir.

**Methods:** An open-label, single-centre, randomized controlled trial conducted in adult virologically suppressed HIV-infected patients on triple therapy (TT) with elvitegravir-cobicistat, emtricitabine and tenofovir alafenamide or dolutegravir (DTG), abacavir, and lamivudine (3TC). Participants were randomized to continue TT or switch to DTG, or darunavir/cobicistat (DRVc) plus 3TC. IR was assessed by CD4<sup>+</sup>/CD8<sup>+</sup> ratio at 48 and 96 weeks. Changes in immune activation, proliferation, exhaustion, senescence, and apoptosis in CD4<sup>+</sup> and CD8<sup>+</sup> T cells, plasma sCD14, hsCRP, D-dimers, β2-microglobulin, IL-6, TNF-α and IP-10 levels, cell-associated HIV-DNA (CA-DNA), and unspliced HIV-RNA (usRNA) were also analysed.

**Results:** One hundred and fifty-one participants were enrolled. Fourteen patients did not complete the follow up. In the ITT and PP analysis, the IR was similar between the treatment arms. In the ITT analysis, the median increase in CD4<sup>+</sup>/CD8<sup>+</sup> ratio was 0.10, 0.04, and 0.07 at week 48, and 0.09, 0.05, and 0.08 at week 96 for TT, DTG/3TC, and DRVc/3TC, respectively. After adjusting for confounding factors, the slopes of changes in CD4<sup>+</sup>/CD8<sup>+</sup> ratio over time were independent of treatment ( $F = 1.699$ ;  $p = 0.436$ ) and related only to baseline values ( $F = 756.871$ ;  $p = 0.000$ ). There were no differences in IA/I, CA-DNA, or usRNA between treatment arms.

**Discussion:** Both IR and IA/I, CA-DNA, and usRNA were similar in the three treatment groups, regardless of maintaining TT or simplifying to DTG/3TC or DRVc/3TC in virologically suppressed HIV-infected patients. **María Trujillo-Rodríguez, Clin Microbiol Infect 2022;28:1151.e9–1151.e16**

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## Introduction

The gold standard for antiretroviral treatment (ART) has been based on two nucleos(t)ide reverse transcriptase inhibitors (NRTI) combined with a protease inhibitor (PI), non-nucleoside reverse transcriptase inhibitor (NNRTI), or an integrase strand transfer

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inhibitor. The idea of simplifying ART came up because of the short- and long-term toxicity and frequent resistance-associated mutations. However, early simplification strategies failed to maintain viral suppression, due to a low genetic barrier and antiviral activity of drugs [1]. The idea re-emerged in the form of ritonavir-boosted PI monotherapy, although those regimens resulted in more low-level viral replication episodes and virological failures than triple therapy (TT) [2]. Recently, simplification strategies based on a boosted PI or dolutegravir (DTG) plus lamivudine (3TC) have shown similar virological efficacy to TT in HIV-suppressed patients [3–7] and even in treatment-naïve patients [8], with lower cost [9].

On the other hand, although ART reduces the immune activation and inflammation (IA/I), it fails to completely normalize them, despite plasma viral load suppression, playing a pivotal role in non-AIDS events [10]. Incomplete suppression of viral replication in lymphatic tissues, not reflected in plasma, could contribute to it due to lower drug concentrations in tissues [11]. Consequently, there is concern that simplification to dual therapy (DT) could not control viral replication as much as TT, impacting immune recovery (IR), IA/I, or HIV reservoir. To date, there are scarce data on DT that have evaluated partial aspects of these issues, sometimes with contradictory results, and most without control groups [7,12–18]. Moreover, expert opinions believe that DT could negatively affect patients' prognosis [19]. Thus, this randomized clinical trial aimed to provide insights into this controversy through a comprehensive evaluation of IR, IA/I, cell-associated HIV-DNA (CA-DNA), and unspliced HIV-RNA (usRNA) up to 96 weeks of follow up.

## Methods

### Study design and participants

The TRIDUAL (TRiple versus DUAL therapy) study is an open-label, single-centre, noninferiority, randomized clinical trial (ClinicalTrials.gov: NCT03447873) carried out in Virgen del Rocío University Hospital in Seville, Spain.

Eligible participants were adults who started ART later than 01 January 2012, had undetectable plasma HIV RNA for at least 1 year, and were  $\geq 6$  months on FTC/TAF/EVGc or ABC/3TC/DTG. Exclusion criteria included HIV resistance to the study drugs, pregnancy, active opportunistic infection, HBV or HCV coinfection, cirrhosis, portal hypertension, previous or current malignancies, treatment with immunomodulatory agents, and use of drugs with potential interactions with the prescribed drugs.

Participants who met the conditions were centrally randomized via phone interface at the clinical trials unit of our hospital based on time with undetectable viral load (1–2, 2–3, 3–4, or >4 years), to continue with the previous TT or switch to DTG/3TC (50/300 mg) or DRVcobicistat (DRVc)/3TC (800<sub>150</sub>/300 mg), using random numbers and making the relative sizes of the groups in each stratum similar. The randomization list was constructed using the separate strata balanced randomization module of the WinPepi Etcetera program (Epidemiologic Perspectives & Innovations) (see Table S1).

Patients were excluded in case of virological failure, defined as two consecutive plasma viral loads  $\geq 200$  copies/mL, treatment change or interruption, loss of follow up, a diagnosis of malignancy, or death. We selected an arbitrary cut-off point of  $\geq 200$  copies/mL to define virological failure because of possible measurement errors in HIV RNA assays close to their limit of quantification [20].

### Ethics

The study was conducted according to Good Clinical Practice principles after being approved by the Ethics Committee for Clinical Research of the Virgen Macarena and Virgen del Rocío University Hospitals (03/2017) and the National Health Authority. All participants provided signed informed consent.

### Endpoints, follow up, and assessments

Patients were assessed at baseline, at the third and sixth month, and then every 6 months, including adverse effects, haematology and biochemistry tests, CD4<sup>+</sup> and CD8<sup>+</sup> T cell counts in fresh blood with an FC 500 flow cytometer (Beckman Coulter, Brea, CA), and plasma HIV RNA levels (Cobas AmpliPrep-Cobas TaqMan HIV-1 test, version 2.0; Roche Diagnostics) with a lower detection limit of 20 copies per milliliter.

The primary endpoint was to evaluate IR at 48 weeks after switching to DT compared with continuing on TT. Secondary endpoints were IR at 96 weeks, changes in IA/I markers, CA-DNA, and usRNA at 48 and 96 weeks. IR was assessed by CD4<sup>+</sup>/CD8<sup>+</sup> ratio. Activated (CD38<sup>+</sup>HLA-DR<sup>+</sup>), proliferating (Ki67<sup>+</sup>), exhausted (PD-1<sup>+</sup>), senescent (CD57<sup>+</sup>CD28<sup>-</sup>), and apoptotic (annexin V<sup>+</sup>) CD4<sup>+</sup> and CD8<sup>+</sup> were also identified. We measured plasma sCD14 as monocyte activation marker, hsCRP, D-dimers,  $\beta 2M$ , IL-6, TNF- $\alpha$ , and IP-10 as markers of inflammation and coagulation, and CA-DNA and usRNA as HIV reservoir and transcription markers.

Methods for cell surface staining for immune profile, quantitation of cell-associated HIV-DNA and usHIV-RNA, and enzyme-linked immunosorbent assays are described in the Supplementary material.

### Statistical analysis

Given that we had not found clear references in the literature to calculate the sample size and the proof-of-concept nature of this study, no formal sample size was calculated. It was based on a retrospective analysis of our database and feasibility resources. We aimed to include 50 participants per treatment arm, which could provide enough information. In addition, a post-hoc analysis was performed to calculate statistical power [21].

Results were expressed as medians and IQRs for continuous variables and numbers and percentages for categorical variables. The Kruskal-Wallis test and the Friedman test was used to compare changes in quantitative and continuous variables over time in each treatment group, respectively. Correlations were assessed by Spearman's correlation coefficient.

A linear mixed model with random intercepts and slopes for repeated measurements was used to compare the time courses of the CD4<sup>+</sup>/CD8<sup>+</sup> ratio according to regimen strategy, adjusted for possible confounders, including age, sex, baseline values, nadir CD4<sup>+</sup>, and time with undetectable viral load. The overall effect of each explanatory variable on the outcome variable CD4<sup>+</sup>/CD8<sup>+</sup> ratio was tested with the F-test and CI<sub>95</sub>. A missing data analysis was conducted. Expectation maximization algorithms were applied to identify the nature of missingness (MCAR [Missing completely at random] or MAR [Missing at random]), by Little's Missing MCAR Test. The multiple imputation method was used for dealing with missing data [22].

Analyses were performed in the intention-to-treat (ITT) population and per-protocol (PP). The ITT population included all participants who underwent randomization. In the PP analysis, only

those participants who completed the follow up of the study were analysed.

SPSS v. 26.0 and R software were used for statistical analyses; *p* values < 0.05 were considered significant.

## Results

### Study population

One hundred and fifty-one participants were enrolled between May 2017 and September 2018 (ITT population); demographics and HIV infection data are detailed in Table 1. Fifty-three patients were randomized to continue TT, 50 to DTG/3TC, and 48 to DRVc/3TC. Regarding the time with undetectable plasma viral load, 41 (27.2%) patients were between 1 and 2 years, 40 (26.5%) between 2 and 3, 21 (13.9%) between 3 and 4, and 49 (32.5%) >4 years without differences among groups (*p* = 0.346) (see Fig. S1).

Eight (5.3%) patients were withdrawn from the study before completing 48 weeks, and a further six patients during the second year. Therefore, 137 (90.7%) participants formed the per-protocol population (Fig. 1). Only one virological failure (2370 copies/mL) was observed in the DRVc/3TC arm, with a self-reported full ART adherence, and without resistance mutations detected.

No patient had adverse effects or significant laboratory abnormalities (data not shown).

### Immune recovery

In the ITT analysis, there were no differences in the CD4<sup>+</sup>/CD8<sup>+</sup> ratio baseline between groups (TT, 1.0 [0.7–1.4]; DTG/3TC, 0.9 [0.6–1.2]; DRVc/3TC, 0.9 [0.6–1.2]) (*p* = 0.173). According to the treatment arm, the increase in the CD4<sup>+</sup>/CD8<sup>+</sup> ratio at week 48 were 0.10 (–0.01 to 0.25), 0.04 (–0.03 to 0.19), and 0.07 (0–0.23) for TT, DTG/3TC, and DRVc/3TC (*p* = 0.318), respectively. From baseline to week 96, these increases were 0.09 (–0.02 to 0.25), 0.05 (–0.03 to 0.15), and 0.08 (0–0.21) (*p* = 0.378), respectively. In PP analysis, these figures were 0.09 (–0.01 to 0.25), 0.04 (–0.03 to 0.20), and

0.07 (0–0.24) for TT, DTG/3TC, and DRVc/3TC (*p* = 0.435), respectively, at week 48 and 0.09 (–0.02 to 0.25), 0.05 (–0.03 to 0.15), and 0.08 (0–0.21) for TT, DTG/3TC, and DRVc/3TC (*p* = 0.378), respectively, at week 96. Baseline and evolutive data for each treatment arm are shown in Table 2.

A lineal mixed model adjusted for age, sex, baseline CD4<sup>+</sup>/CD8<sup>+</sup> ratio, time with undetectable viral load, and treatment × time interaction showed that the slopes of change over time for CD4<sup>+</sup>/CD8<sup>+</sup> ratio were independent of treatment and related only to baseline values both by ITT (Table 3 and Fig. 2) and PP analysis (see Fig. S2 and Table S2), respectively.

The baseline CD4<sup>+</sup>/CD8<sup>+</sup> ratio correlated inversely with activation (*ρ* = –0.406), proliferation (*ρ* = –0.380) and exhaustion (*ρ* = –0.352) of CD4<sup>+</sup> T cells (all, *p* < 0.0001), but most of them disappeared throughout follow up. Likewise, a negative relationship between CD4<sup>+</sup>/CD8<sup>+</sup> ratio and usRNA levels was observed during the follow up (*ρ* = –0.288 at week 48 and –0.282 at week 96; all *p* < 0.0001). Nevertheless, inconsistent associations were detected between CD4<sup>+</sup>/CD8<sup>+</sup> ratio with soluble factors and CA-DNA (see S3–S5).

### Immune activation and inflammation

During the follow up there were no differences between the treatment arms in activation, proliferation, exhaustion, senescence, and apoptosis of CD4<sup>+</sup> and CD8<sup>+</sup> T cells. Detailed data by treatment arm are described in Table 2.

On the other hand, the sCD14 decreased (*p* ≤ 0.002) and IL-6 had a small increase (*p* < 0.0001) regardless of the treatment arm and without differences among them (Table 2). Besides, β2M, hsCRP, D-dimers, TNF-α, and IP-10 levels showed small fluctuations without differences between groups.

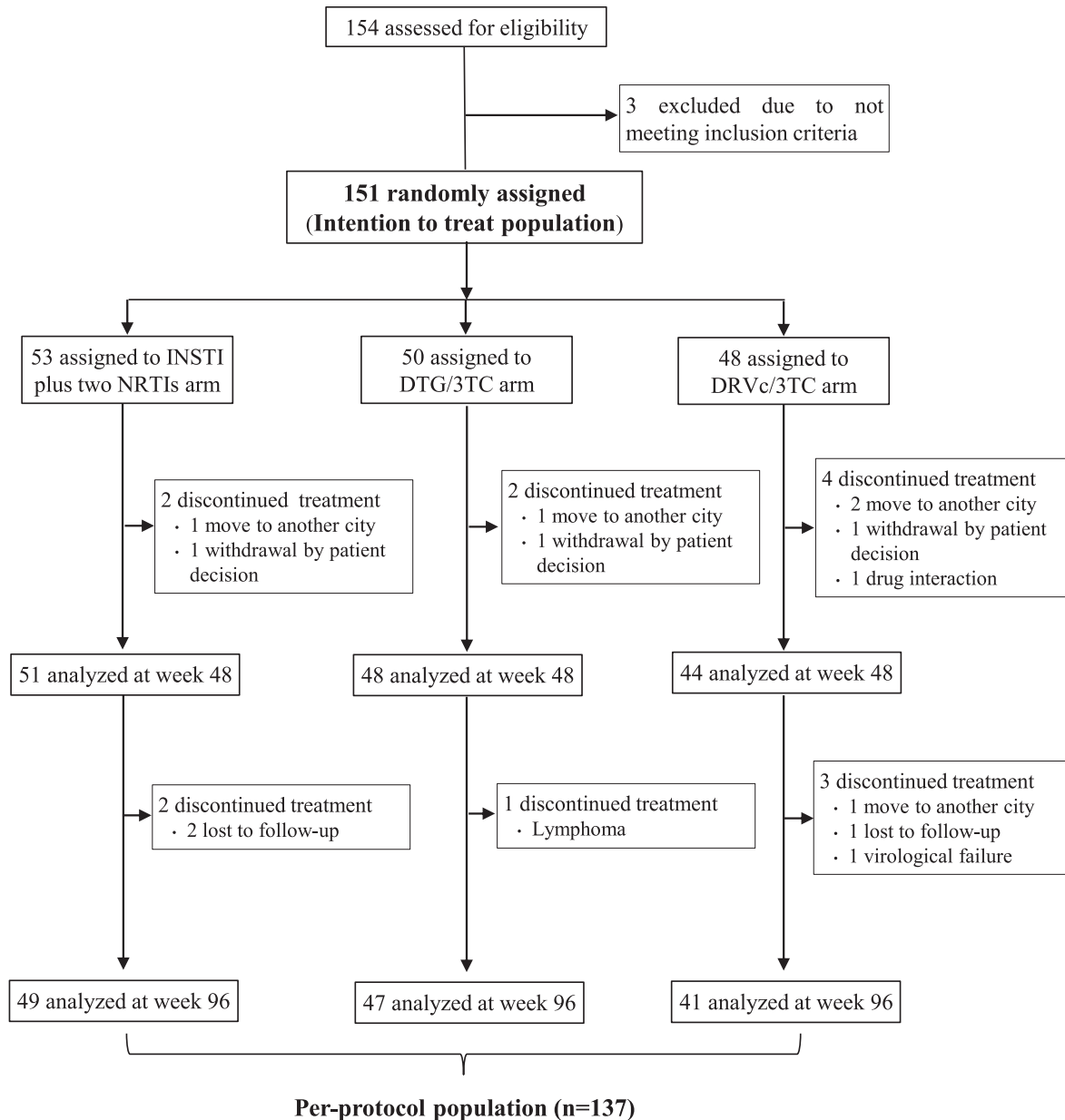
### HIV reservoir and transcriptional activity

CA-DNA did not change during the first year (*p* = 0.474) but decreased during the second year a median of 0.11 log<sub>10</sub> (*p* = 0.02), without differences in treatment groups (*p* = 0.659).

**Table 1**  
Baseline patient characteristics of ITT population

	INSTI +2 NRTIs (n = 53)	DTG/3TC (n = 50)	DRVc/3TC (n = 48)
Male sex	51 (96.2)	44 (88.0)	44 (91.7)
Age, years	33 (28–44)	29 (26–37)	34 (25–41)
Age group			
<30 years	15 (28.3)	26 (52.0)	16 (33.0)
30–45 years	29 (54.7)	18 (36.0)	25 (52.1)
>45 years	9 (17.0)	6 (12.0)	7 (14.6)
Risk factor for HIV			
MSM	44 (83.0)	38 (76.0)	36 (75.0)
Heterosexual	9 (17.0)	7 (14.0)	7 (14.6)
Other/Unknown		2 (4.0)	4 (8.3)
IVDU		3 (6.0)	1 (2.1)
Previous CDC C stage	2 (3.8)	3 (6.0)	4 (8.3)
Nadir CD4 count, cells/μL	342 (266–453)	282 (191–416)	284 (144–403)
Nadir CD4 <sup>+</sup> /CD8 <sup>+</sup> ratio	0.4 (0.2–0.5)	0.3 (0.2–0.5)	0.2 (0.2–0.5)
Months on treatment	37 (23–62)	38 (26–61)	39 (29–73)
HIV-RNA <50 copies/mL, months	31 (18–56)	34 (23–57)	34 (24–70)
HIV-RNA <50 copies/mL group			
1–2 years	17 (32.1)	12 (24.0)	12 (25.0)
2–3 years	14 (26.4)	14 (28.0)	12 (25.0)
3–4 years	5 (9.4)	9 (18.0)	7 (14.6)
>4 years	17 (32.1)	15 (30.0)	17 (35.4)
Baseline CD4 count, cells/μL	794 (596–1123)	750 (590–917)	711 (542–976)
Baseline CD4 <sup>+</sup> /CD8 <sup>+</sup> ratio	1.0 (0.7–1.4)	0.9 (0.6–1.1)	0.8 (0.6–1.2)

Data are expressed as median (interquartile range) or n (%). INSTI, integrase inhibitor; NRTIs, nucleos(t)ide reverse transcriptase inhibitor; DRVc, darunavir/cobicistat; DTG, dolutegravir; 3TC, lamivudine; MSM, men who have sex with men; IVDU, previous intravenous drug use.



**Fig. 1.** Study flow diagram. INSTI, integrase inhibitor; NRTIs, nucleos(t)ide reverse transcriptase inhibitor; DRVc, darunavir/cobicistat; DTG, dolutegravir; 3TC, lamivudine; ART, antiretroviral treatment.

Baseline and evolutive data for each treatment arm are shown in [Table 2](#).

The most consistent association found with CA-DNA was the CD4<sup>+</sup> nadir at baseline and week 48 ( $\rho = -0.292$ , and  $-0.328$ , respectively; both  $p < 0.0001$ ) (see [Figs. S3–S5](#)). Conversely, usRNA increased a median of 0.3 log<sub>10</sub> during the second year, equally in all three groups ( $p = 0.223$ ). CA-DNA and usRNA levels were strongly correlated throughout follow up ( $\rho = 0.332$  at baseline, 0.521 at week 48, and 0.362 at week 96; all  $p < 0.0001$ ). At baseline and 48 weeks, the usRNA levels were associated positively with activation, proliferation, exhaustion, and apoptosis of CD4<sup>+</sup> T cells, although this relationship disappeared at week 96. In addition, correlations between usRNA levels with activation, proliferation, exhaustion, and senescence of CD8<sup>+</sup> T cells were observed at baseline, although there were not long-term associations (see [Figs. S3–S5](#)).

## Discussion

This study has not detected differences in IR, IA/I, HIV reservoir, nor the transcriptional activity in virologically suppressed patients regardless of maintaining TT or switching to DT.

To date, only partial aspects of simplification's immunological or inflammatory consequences of DT have been evaluated in virologically suppressed patients. Quirós-Roldan et al. observed that CD4<sup>+</sup>/CD8<sup>+</sup> ratio continued to rise after simplification to boosted atazanavir or DRV plus 3TC [12]. Monsalvo et al. observed that the CD4<sup>+</sup>/CD8<sup>+</sup> ratio increase of 245 patients who switched to boosted DRV or DTG based NRTI-sparing therapies over 48 weeks was only relative to baseline values [18]. These data are similar to our results after 48 and 96 weeks by ITT and PP.

Maggiolo et al. have explored CD8<sup>+</sup> activation after switching to boosted DRV plus rilpivirine versus maintaining a boosted PI plus

**Table 2**  
Evolution of immune activation, inflammation, and HIV reservoir parameters over follow up in patients with INSTI + 2 NRTIs, DTG/3TC, and DRVc/3TC of ITT population

	INSTI + 2 NRTIs			Basal (n = 50)	DTG/3TC		DRVc/3TC			<sup>a</sup> p	<sup>b</sup> p
	Basal (n = 53)	Week 48 (n = 51)	Week 96 (n = 49)		Week 48 (n = 48)	Week 96 (n = 47)	Basal (n = 48)	Week 48 (n = 44)	Week 96 (n = 41)		
<b>CD4<sup>+</sup>/CD8<sup>+</sup> ratio</b>	1.0 (0.7–1.4)	1.1 (0.7–1.6)	1.1 (0.8–1.6)	0.9 (0.6–1.1)	1.0 (0.7–1.3)	0.9 (0.6–1.2)	0.8 (0.6–1.2)	1.1 (0.8–1.3)	1.0 (0.7–1.3)	0.414	0.218
<b>CD4<sup>+</sup> T cells</b>											
HLA-DR <sup>+</sup> CD38 <sup>+</sup> (%)	1.2 (1.0–1.8)	1.2 (0.9–1.7)	1.1 (0.6–1.9)	1.5 (1.1–1.8)	1.4 (1.1–2.4)	1.5 (1.0–2.4)	1.7 (1.2–2.2)	1.4 (1.2–2.0)	1.7 (1.0–2.9)	0.086	0.333
Ki67 <sup>+</sup> (%)	0.7 (0.5–1.2)	0.9 (0.6–1.4)	1.2 (0.8–2.3)	0.8 (0.6–1.3)	0.9 (0.6–1.5)	1.7 (1.2–2.6)	0.9 (0.5–1.5)	0.9 (0.6–1.1)	2.0 (1.2–2.5)	0.307	0.446
PD-1 <sup>+</sup> (%)	7.1 (5.0–12.7)	6.7 (4.4–9.9)	9.2 (5.9–17.3)	8.7 (6.8–12.9)	6.8 (5.2–10.9)	12.9 (6.4–18.2)	7.2 (5.4–11.9)	5.8 (4.1–8.3)	10.5 (5.5–15.8)	0.605	0.314
CD28 <sup>-</sup> CD57 <sup>+</sup> (%)	5.6 (1.8–12.1)	6.8 (2.8–19.1)	3.4 (1.2–7.5)	3.1 (1.5–8.9)	8.1 (1.4–13.9)	2.6 (1.4–7.8)	4.7 (2.5–8.9)	5.7 (2.9–9.1)	4.9 (2.8–7.4)	0.236	0.863
Annexin V <sup>+</sup> (%)	0.6 (0.4–1.2)	0.9 (0.5–1.5)	2.2 (1.7–2.9)	0.8 (0.5–1.5)	0.9 (0.7–1.5)	2.0 (1.3–2.4)	0.7 (0.4–1.3)	1.2 (0.7–1.6)	2.1 (1.7–2.9)	0.529	0.316
<b>CD8<sup>+</sup> T cells</b>											
HLA-DR <sup>+</sup> CD38 <sup>+</sup> (%)	1.7 (1.1–2.6)	1.4 (0.8–2.2)	1.1 (0.6–2.0)	1.7 (1.0–2.9)	1.5 (0.9–2.4)	1.5 (0.9–2.3)	2.2 (1.6–3.9)	1.5 (1.1–2.5)	1.6 (0.9–2.4)	0.782	0.573
Ki67 <sup>+</sup> (%)	0.7 (0.4–1.0)	0.6 (0.3–0.9)	1.4 (0.9–1.9)	0.6 (0.4–0.9)	0.6 (0.4–0.9)	1.6 (1.0–2.1)	0.7 (0.4–1.0)	0.6 (0.4–0.8)	1.6 (1.4–2.0)	0.471	0.426
PD-1 <sup>+</sup> (%)	8.2 (4.3–12.9)	7.9 (4.1–10.6)	13.6 (6.4–19.7)	8.2 (5.1–13.1)	8.0 (4.9–11.1)	12.9 (8.7–18.9)	7.8 (3.9–10.6)	6.2 (3.9–9.9)	9.9 (5.6–16.4)	0.801	0.297
CD28 <sup>-</sup> CD57 <sup>+</sup> (%)	41.5 (30.6–51.9)	47.9 (36.1–57.9)	37.5 (24.8–49.7)	39.2 (33.2–46.6)	48.7 (33.5–59.7)	33.9 (25.7–40.9)	40.6 (32.9–51.9)	40.5 (29.5–53.9)	36.7 (28.2–49.6)	0.122	0.874
Annexin V <sup>+</sup> (%)	0.9 (0.7–1.8)	1.0 (0.6–1.7)	2.4 (1.7–2.8)	1.1 (0.7–2.2)	1.0 (0.7–1.7)	2.1 (1.4–2.6)	0.9 (0.5–1.6)	1.1 (0.8–1.6)	2.2 (1.5–2.7)	0.071	0.525
β2 microglobulin (mg/L)	1.9 (1.7–2.2)	1.9 (1.7–2.2)	2.0 (1.8–2.3)	1.9 (1.7–2.3)	1.9 (1.7–2.3)	2.0 (1.7–2.3)	2.1 (1.8–2.3)	1.9 (1.8–2.2)	2.1 (1.8–2.4)	0.886	0.744
sCD14 (μg/mL)	2.7 (1.9–3.6)	2.5 (1.7–3.5)	1.5 (1.3–1.9)	3.0 (2.1–3.6)	2.3 (1.6–3.5)	1.5 (1.2–1.8)	2.7 (1.8–3.6)	2.2 (1.8–3.1)	1.7 (1.2–1.9)	0.832	0.506
hsCRP (mg/L)	1.8 (0.9–3.6)	1.9 (0.9–3.9)	1.3 (0.7–3.2)	1.1 (0.7–2.9)	1.2 (0.7–2.0)	1.2 (0.6–3.4)	1.3 (0.7–2.6)	1.2 (0.7–1.9)	1.7 (0.9–2.9)	0.468	0.438
IL-6 (pg/mL)	1.7 (1.3–2.3)	1.7 (1.1–2.8)	2.5 (1.6–3.9)	1.9 (1.2–2.7)	1.9 (1.2–3.3)	2.5 (1.6–4.7)	1.7 (1.1–2.9)	1.7 (1.3–2.9)	2.5 (1.7–4.7)	0.390	0.982
TNF-α (pg/mL)	0.9 (0.6–1.2)	0.9 (0.6–1.3)	0.7 (0.6–0.9)	0.8 (0.7–1.2)	0.9 (0.7–1.2)	0.8 (0.6–1.1)	0.9 (0.8–1.2)	0.9 (0.8–1.2)	0.8 (0.7–1.2)	0.535	0.988
IP-10 (pg/mL)	81.4 (56.4–126.4)	76.2 (57.0–113.5)	77.2 (54.7–90.6)	81.2 (55.9–114.5)	86.3 (55.3–113.5)	80.3 (57.5–110.3)	100.7 (73.3–134.4)	94.1 (51.8–136.5)	81.7 (60.1–112.9)	0.553	0.195
D-Dimers (μg/L)	255.0 (200.0–372.5)	225.0 (170.0–305.0)	240.0 (185.0–305.0)	280.0 (220.0–350.0)	220.0 (170.0–370.0)	270.0 (220.0–382.5)	260.0 (205.0–367.5)	245.0 (170.0–347.5)	220.0 (190.0–295.0)	0.915	0.161
HIV-DNA log <sub>10</sub> copies /10 <sup>6</sup> PBMC	2.6 (2.3–3.0)	2.6 (2.4–2.9)	2.4 (2.3–2.6)	2.6 (2.4–2.9)	2.7 (2.6–2.9)	2.5 (2.2–2.7)	2.6 (2.4–2.9)	2.6 (2.3–2.9)	2.5 (2.2–2.9)	0.715	0.659
usHIV-RNA log <sub>10</sub> copies /10 <sup>6</sup> TBP	2.8 (2.5–3.1)	2.9 (2.6–3.3)	3.1 (2.8–3.6)	2.9 (2.7–3.3)	3.0 (2.7–3.2)	3.3 (3.0–3.6)	3.0 (2.4–3.3)	3.0 (2.5–3.5)	3.2 (2.6–3.7)	0.204	0.223

Data are expressed as median (IQR). INSTI, integrase inhibitor; NRTIs, nucleos(t)ide reverse transcriptase inhibitor; DTG, dolutegravir; 3TC, lamivudine; DRVc, darunavir/cobicistat.

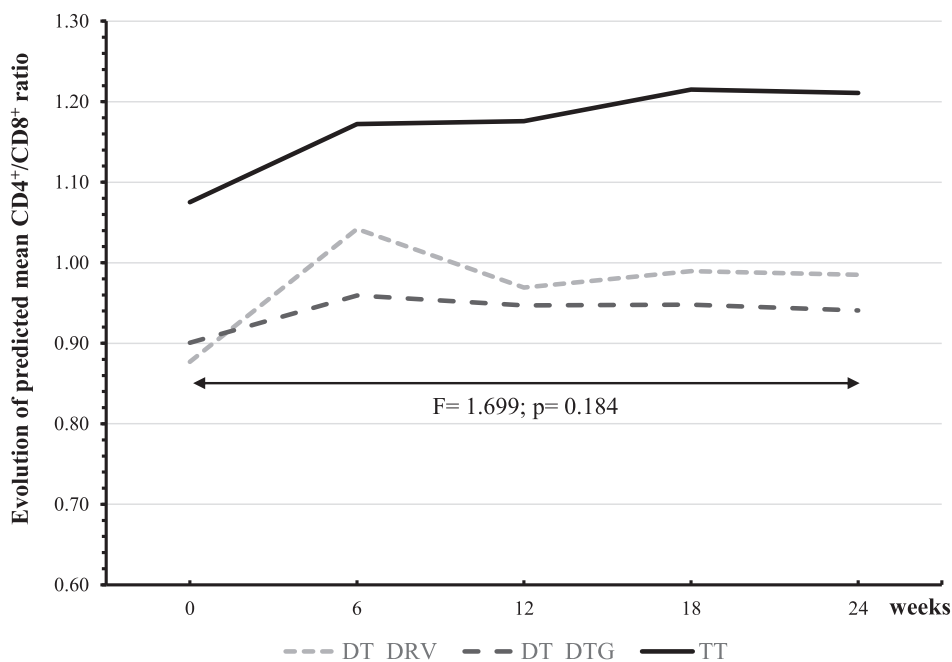
<sup>a</sup> p value for differences between baseline and week 48 among IT, DTG/3TC, and DRVc/3TC treatment arms.

<sup>b</sup> p value for differences between baseline and week 96 among IT, DTG/3TC, and DRVc/3TC treatment arms.

**Table 3**  
Fixed effects on the CD4<sup>+</sup>/CD8<sup>+</sup> ratio by ITT analysis

Evolution of the CD4 <sup>+</sup> /CD8 <sup>+</sup> ratio					
Fixed effects	Estimate	95% CI	F	p	Post-hoc power (%)
Intercept	0.1024	[-0.1124–0.3173]	3.472	0.064	45.77
Time with plasma HIV-RNA <50 copies/mL (years)			1.755	0.158	41.12
1–2	0.0789	[-0.0046–0.1626]		0.064	
2–3	-0.0088	[-0.0923–0.0747]		0.835	
3–4	0.0483	[-0.0538–0.1506]		0.351	
>4	ref.				
Sex			0.057	0.811	5.65
Men	0.0147	[-0.1069–0.1364]		0.811	
Women	ref.				
Age	-0.0004	[-0.0038–0.0029]	0.063	0.801	5.72
Baseline CD4 <sup>+</sup> /CD8 <sup>+</sup> ratio	0.9456	[0.8779–1.0134]	756.871	0.000	100
Time (months)	0.0193	[-0.0022–0.0409]	0.608	0.436	12.17
ART regimen			1.699	0.184	35.73
INSTI + 2 NRTIs	ref.				
DTG/3TC	-0.0409	[-0.1519–0.0699]		0.468	
DRVc/3TC	0.0630	[-0.0483–0.1744]		0.267	
ART regimen x time			1.552	0.213	32.94
INSTI + 2 NRTIs x time	ref.				
DTG/3TC x time	-0.0147	[-0.0457–0.0162]		0.350	
DRVc/3TC x time	-0.0280	[-0.0593–0.0032]		0.079	

ref., reference category; INSTI, integrase inhibitor; NRTIs, nucleos(t)ide reverse transcriptase inhibitor; DRVc, darunavir/cobicistat; DTG, dolutegravir; 3TC, lamivudine.



**Fig. 2.** Evolution of the slopes of change over time for the CD4<sup>+</sup>/CD8<sup>+</sup> ratio in the three treatments arms by ITT analysis. The slopes are adjusted by the estimated beta coefficients of the specific confounders and covariates for the model: age, sex, baseline values, nadir CD4<sup>+</sup>, time with undetectable viral load, and ART regimen × time interaction. INSTI, integrase inhibitor; NRTIs, nucleos(t)ide reverse transcriptase inhibitor; DRVc, darunavir/cobicistat; DTG, dolutegravir; 3TC, lamivudine.

two NRTIs, with a decrease in CD8<sup>+</sup>CD38<sup>+</sup>HLA-DR<sup>+</sup> cells in both arms [23]. Our results showed slight fluctuations in IA, exhaustion, proliferation, senescence, and apoptosis on CD4<sup>+</sup> and CD8<sup>+</sup> T cells over 96 weeks of follow up, but without differences between the three regimens.

Regarding the plasma inflammation and coagulation markers, Vallejo et al. in a cross-study observed that sCD14 and IL-6 levels in DT participants were lower than those on TT [24]. Likewise, Belmonti et al. in a substudy of the ATLAS-M trial reported that simplification to boosted atazanavir plus 3TC did not affect inflammation markers compared with maintaining TT [16]. Finally,

in the phase 3 SWORD-1&2 trial, Aboud et al. did not find a consistent pattern of change in inflammation and coagulation markers between patients on DTG/rilpivirine and those on TT after 48 weeks [17], consistent with our results, except that we detected a significant decrease in sCD14 in all treatment arms. However, Serrano-Villar et al. suggested that reducing ART to less than three drugs may lead to a less favorable long-term anti-inflammatory profile (Serrano-Villar et al., 23rd International Aids Conference 2020). In our study, we observed a small increase in IL-6 levels in all treatment groups. The mechanisms involved in the increase of IL-6 may be complex, although we have not been able to identify which

factor or factors may be responsible for this. In any case, it is reassuring that it occurred equally in all three treatment groups and is a further reason to continue to follow these patients in the longer term.

HIV reservoir has only been evaluated in two studies by Lombardi et al., who observed, just like us, a similar decline in HIV-DNA levels with DT and TT [15,25].

Finally, we evaluated for the first time the impact of simplification to DT on HIV transcription. We detected a slight increase in usRNA, with no differences between groups. At both baseline and week 48, usRNA levels were associated with the activation, proliferation, exhaustion, and apoptosis of CD4<sup>+</sup> T cells. Based on our results, we hypothesize that usRNA may be one of the causes of the persistent phenotypic alterations of CD4<sup>+</sup> T cells.

Our study has some limitations. First, the analyses are based on PBMC, (peripheral blood mononuclear cells), which may not always reflect tissue-related processes during HIV infection. Second, the assumption made for sample size estimate may not be entirely accurate.

Since we have not detected differences in IR, IA/I, or HIV reservoir between maintaining an integrase strand transfer inhibitor-based TT or simplifying to DTG or DRVc plus 3TC in virologically suppressed HIV-infected patients, we can conclude that dual therapy is a suitable option for antiretroviral treatment simplification.

### Transparency declaration

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### Author contributions

LFLC was head of the project and AGV developed the protocol. LFLC, MD, GRV, CR, and NE contributed to the recruitment and management of patients. MTR, EMM, ASG, AIAR, LHH, and CL performed the laboratory determinations. LFLC, AGV, MTR, YMG, and JMPF analysed the data. LFLC, AGV, and MTR verified the underlying data and wrote the final manuscript. AGV and LFLC contributed equally to the work. All authors reviewed the manuscript, suggested edits, and approved the final version.

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### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cmi.2022.02.041>.

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