Clinical Microbiology and Infection 28 (2022) 1151.e9–1151.e16

Contents lists available at ScienceDirect

Clinical Microbiology and Infection

journal homepage: www.clinicalmicrobiologyandinfection.com

Original article

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

María Trujillo-Rodríguez [1], Esperanza Muñoz-Muela [1], Ana Serna-Gallego [1], Yusnelkis Milanés-Guisado [1], Juan Manuel Praena-Fernández [2], Ana Isabel Álvarez-Ríos [3], Laura Herrera-Hidalgo [1, 4], Montserrat Domínguez [1], Carmen Lozano [1], Gloria Romero-Vazquez [1], Cristina Roca [1], Nuria Espinosa [1], Alicia Gutiérrez-Valencia [1, *], Luis F. López-Cortés [1]

[1] *Enfermedades Infecciosas, Microbiología Clínica y Medicina Preventiva, Instituto de Biomedicina de Sevilla/Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, Spain*
[2] *Departamento de Bioestadística, Facultad de Medicina, Universidad de Granada, Granada, Spain*
[3] *Departamento de Bioquímica Clínica, Hospital Universitario Virgen del Rocío/CSIC/SAS/Universidad de Sevilla, Spain*
[4] *Farmacia Hospitalaria, Instituto de Biomedicina de Sevilla/Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, Spain*

ARTICLE INFO

Article history:
Received 8 October 2021
Received in revised form
24 February 2022
Accepted 27 February 2022
Available online 11 March 2022

Editor: A. Huttner

Keywords:
Dual therapy
HIV reservoir
HIV treatment
Immune activation and inflammation
Immune recovery
Simplification strategy
Triple therapy

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 $CD4^+/CD8^+$ ratio at 48 and 96 weeks. Changes in immune activation, proliferation, exhaustion, senescence, and apoptosis in $CD4^+$ and $CD8^+$ 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 $CD4^+/CD8^+$ 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 $CD4^+/CD8^+$ 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**

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

* Corresponding author. Alicia Gutiérrez Valencia, Instituto de Biomedicina de Sevilla/Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla. Campus Hospital Universitario Virgen del Rocío. Calle Antonio Maura Montaner s/n., 41013, Sevilla, Spain.
E-mail address: alicia.gutierrez.valencia@gmail.com (A. Gutiérrez-Valencia).

https://doi.org/10.1016/j.cmi.2022.02.041

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 (Clinical trials.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 ≥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_{150}/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 ≥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 ≥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, $CD4^+$ and $CD8^+$ 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 $CD4^+/CD8^+$ ratio. Activated ($CD38^+HLA\text{-}DR^+$), proliferating ($Ki67^+$), exhausted ($PD\text{-}1^+$), senescent ($CD57^+CD28^-$), and apoptotic (annexin V^+) $CD4^+$ and $CD8^+$ were also identified. We measured plasma sCD14 as monocyte activation marker, hsCRP, D-dimers, β2M, IL-6, TNF-α, 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 $CD4^+/CD8^+$ ratio according to regimen strategy, adjusted for possible confounders, including age, sex, baseline values, nadir $CD4^+$, and time with undetectable viral load. The overall effect of each explanatory variable on the outcome variable $CD4^+/CD8^+$ ratio was tested with the F-test and CI_{95}. 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 $CD4^+/CD8^+$ 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 $CD4^+/CD8^+$ 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 $CD4^+/CD8^+$ ratio, time with undetectable viral load, and treatment × time interaction showed that the slopes of change over time for $CD4^+/CD8^+$ 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 $CD4^+/CD8^+$ ratio correlated inversely with activation (ρ = −0.406), proliferation (ρ = −0.380) and exhaustion (ρ = −0.352) of $CD4^+$ T cells (all, p < 0.0001), but most of them disappeared throughout follow up. Likewise, a negative relationship between $CD4^+/CD8^+$ 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 $CD4^+/CD8^+$ 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 $CD4^+$ and $CD8^+$ 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 $\log_{10}$ (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 $CD4^+/CD8^+$ 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 $CD4^+/CD8^+$ 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.

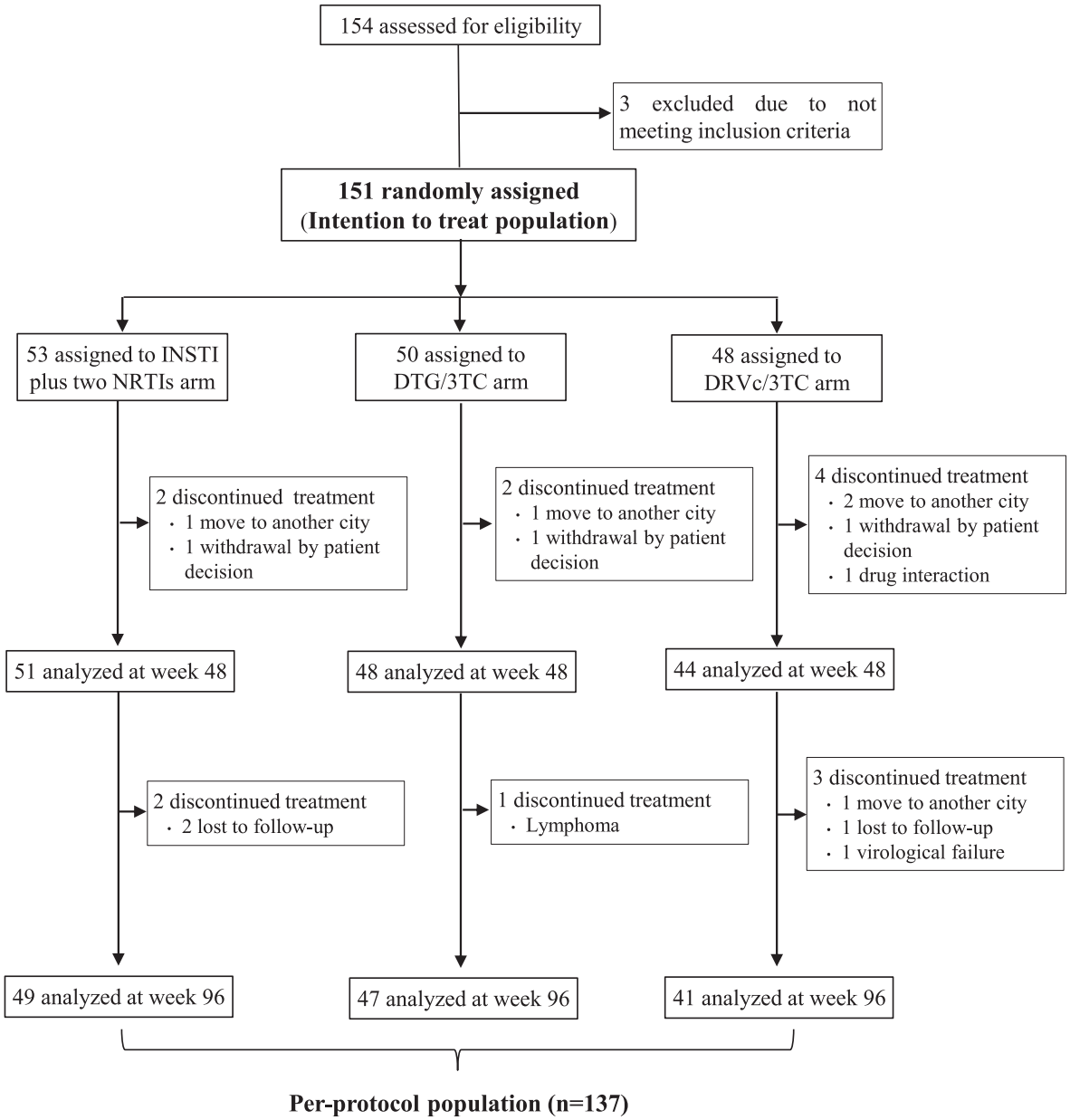

Fig. 1. Study flow diagram. INSTI, integrase inhibitor; NRTIs, nucleos(t)ide reverse transcriptase inhibitor; DRVc, darunavir/cobicistat; DTG, dolutegravir; 3TC, lamivudine; ART, antiretroviral treatment.

Baseline and evolutive data for each treatment arm are shown in Table 2.

The most consistent association found with CA-DNA was the $CD4^+$ 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 $\log_{10}$ 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 $CD4^+$ T cells, although this relationship disappeared at week 96. In addition, correlations between usRNA levels with activation, proliferation, exhaustion, and senescence of $CD8^+$ 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 $CD4^+/CD8^+$ ratio continued to rise after simplification to boosted atazanavir or DRV plus 3TC [12]. Monsalvo et al. observed that the $CD4^+/CD8^+$ 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 $CD8^+$ 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			DTG/3TC		DRVc/3TC			[a] p	[b] p
	Basal ($n = 53$)	Week 48 (n = 51)	Week 96 (n = 49)	Basal ($n = 50$)	Week 48 ($n = 48$)	Week 96 ($n = 47$)	Basal ($n = 48$)	Week 48 ($n = 44$)	Week 96 ($n = 41$)		
$CD4^+/CD8^+$ ratio	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
$CD4^+$ T cells											
HLA-DR^+CD38^+ (%)	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
$Ki67^+$ (%)	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
$PD\text{-}1^+$ (%)	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
$CD28^-CD57^+$ (%)	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^+ (%)	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
$CD8^+$ T cells											
HLA-DR^+CD38^+ (%)	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
$Ki67^+$ (%)	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
$PD\text{-}1^+$ (%)	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
$CD28^-CD57^+$ (%)	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^+ (%)	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_{10} copies $/10^6$ 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_{10} copies $/10^6$ 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.
[a] p value for differences between baseline and week 48 among TT, DTG/3TC, and DRVc/3TC treatment arms.
[b] p value for differences between baseline and week 96 among TT, DTG/3TC, and DRVc/3TC treatment arms.

Table 3
Fixed effects on the $CD4^+/CD8^+$ ratio by ITT analysis

Evolution of the $CD4^+/CD8^+$ 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 $CD4^+/CD8^+$ 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.

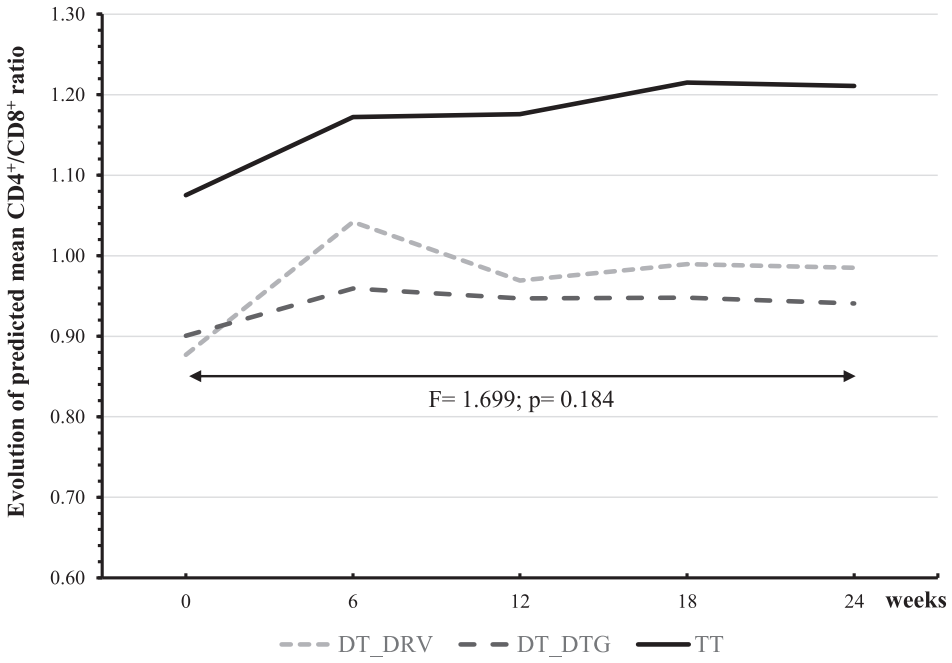

Fig. 2. Evolution of the slopes of change over time for the $CD4^+/CD8^+$ ratio in the three treatments arms by ITT analysis. The slopes are adjusted by the estimated beta coefficients of the specific confounders and covariates for the model: age, sex, baseline values, nadir $CD4^+$, time with undetectable 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 $CD8^+CD38^+HLA\text{-}DR^+$ cells in both arms [23]. Our results showed slight fluctuations in IA, exhaustion, proliferation, senescence, and apoptosis on $CD4^+$ and $CD8^+$ 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 $CD4^+$ T cells. Based on our results, we hypothesize that usRNA may be one of the causes of the persistent phenotypic alterations of $CD4^+$ 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

LFLC has received unrestricted research funding from Gilead Sciences, Janssen-Cilag, Merck Sharp & Dohme, and ViiV Healthcare, and consultancy fees and lecture fees from Gilead Sciences, Janssen-Cilag, and ViiV Healthcare, outside the submitted work. All other authors declare no competing interests. This work was supported by Instituto de Salud Carlos III through the project (PI18/01298) to LFLC; Subprogram Miguel Servet (CP19/00159) to AGV; PFIS contracts (FI19/00304) to EMM; and Subprograma Rio Hortega (CM19/00152) to LHH; and co-financed by the European Regional Development Fund “a way to make Europe” and The SPANISH AIDS Research Network (RD16/0025/0020-ISCIII-FEDER).

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.

Acknowledgements

This study would not have been possible without the collaboration of all patients, medical and nursing staff, and data managers who have taken part in this project. The findings and conclusions in this paper are those of the authors and do not necessarily represent the funding agencies' official position.

Appendix A. Supplementary data

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

References

[1] Havlir DV, Marschner IC, Hirsch MS, Collier AC, Tebas P, Bassett RL, et al. Maintenance antiretroviral therapies in HIV-infected subjects with undetectable plasma HIV RNA after triple-drug therapy. N Engl J Med 1998;339:1261–8.
[2] Arribas J, Girard P-M, Paton N, Winston A, Marcelin A-G, Elbirt D, et al. Efficacy of protease inhibitor monotherapy vs. triple therapy: meta-analysis of data from 2303 patients in 13 randomized trials. HIV Med 2016;17:358–67.
[3] Gutierrez-Valencia A, García C, Viciana P, Milanés-Guisado Y, Fernandez-Magdaleno T, Espinosa N, et al. No difference in effectiveness of treatment simplification to boosted or unboosted atazanavir plus lamivudine in virologically suppressed in HIV-1-infected patients. PLoS One 2018;13:e0203452.
[4] López-Cortés LF, Castaño MA, López-Ruz MA, Rios-Villegas MJ, Hernández-Quero J, Merino D, et al. Effectiveness of ritonavir-boosted protease inhibitor monotherapy in clinical practice even with previous virological failures to protease inhibitor-based regimens. PLoS One 2016;11:e0148924.
[5] Pulido F, Ribera E, Lagarde M, Pérez-Valero I, Palacios R, Iribarren JA, et al. Dual therapy with darunavir and ritonavir plus lamivudine vs triple therapy with darunavir and ritonavir plus tenofovir disoproxil fumarate and emtricitabine or abacavir and lamivudine for maintenance of human immunodeficiency virus type 1 viral suppression: randomized, open-label, noninferiority DUAL-GESIDA 8014-RIS-EST45 trial. Clin Infect Dis 2017;65:2112–8.
[6] Arribas JR, Girard P-M, Landman R, Pich J, Mallolas J, Martínez-Rebollar M, et al. Dual treatment with lopinavir–ritonavir plus lamivudine versus triple treatment with lopinavir–ritonavir plus lamivudine or emtricitabine and a second nucleos(t)ide reverse transcriptase inhibitor for maintenance of HIV-1 viral suppression (OLE): a randomised, open-label, non-inferiority trial. Lancet Infect Dis 2015;15:785–92.
[7] Maggiolo F, Gulminetti R, Pagnucco L, Digaetano M, Benatti S, Valenti D, et al. Lamivudine/dolutegravir dual therapy in HIV-infected, virologically suppressed patients. BMC Infect Dis 2017;17:215.
[8] Cahn P, Madero JS, Arribas JR, Antinori A, Ortiz R, Clarke AE, et al. Durable efficacy of dolutegravir plus lamivudine in antiretroviral treatment–naive adults with HIV-1 infection: 96-week results from the GEMINI-1 and GEMINI-2 randomized clinical trials. J Acquir Immune Defic Syndr 2020;83:310–8.
[9] Girouard MP, Sax PE, Parker RA, Taiwo B, Freedberg KA, Gulick RM, et al. The cost-effectiveness and budget impact of 2-drug dolutegravir-lamivudine regimens for the treatment of HIV infection in the United States. Clin Infect Dis 2016;62:784–91.
[10] Duffau P, Wittkop L, Lazaro E, le Marec F, Cognet C, Blanco P, et al. Association of immune-activation and senescence markers with non-AIDS-defining comorbidities in HIV-suppressed patients. AIDS 2015;29:2099–108.
[11] Fletcher CV, Staskus K, Wietgrefe SW, Rothenberger M, Reilly C, Chipman JG, et al. Persistent HIV-1 replication is associated with lower antiretroviral drug concentrations in lymphatic tissues. Proc Natl Acad Sci USA 2014;111:2307–12.
[12] Quiros-Roldan E, Magro P, Raffetti E, Izzo I, Borghetti A, Lombardi F, et al. Biochemical and inflammatory modifications after switching to dual antiretroviral therapy in HIV-infected patients in Italy: a multicenter retrospective cohort study from 2007 to 2015. BMC Infect Dis 2018;18:285.
[13] Mussini C, Lorenzini P, Cozzi-Lepri A, Marchetti G, Rusconi S, Gori A, et al. Switching to dual/monotherapy determines an increase in CD8+ in HIV-infected individuals: an observational cohort study. BMC Med 2018;16:79.
[14] Romero-Sánchez MC, Álvarez-Ríos AI, Bernal-Morell E, Genebat M, Vera F, Benhnia MR-E-I, et al. Maintenance of virologic efficacy and decrease in levels of β2-microglobulin, soluble CD40L and soluble CD14 after switching previously treated HIV-infected patients to an NRTI-sparing dual therapy. Antivir Res 2014;111:26–32.
[15] Lombardi F, Belmonti S, Borghetti A, Fabbiani M, Marchetti S, Tamburrini E, et al. Evolution of cellular HIV DNA levels in virologically suppressed patients switching to dolutegravir/lamivudine versus maintaining a triple regimen: a prospective, longitudinal, matched, controlled study. J Antimicrob Chemother 2020;75:1599–603.
[16] Belmonti S, Lombardi F, Quiros-Roldan E, Latini A, Castagna A, Borghetti A, et al. Systemic inflammation markers after simplification to atazanavir/ritonavir plus lamivudine in virologically suppressed HIV-1-infected patients: ATLAS-M substudy. J Antimicrob Chemother 2018;73:1949–54.
[17] Aboud M, Orkin C, Podzamczer D, Bogner JR, Baker D, Khuong-Josses M-A, et al. Efficacy and safety of dolutegravir–rilpivirine for maintenance of virological suppression in adults with HIV-1: 100-week data from the randomised, open-label, phase 3 SWORD-1 and SWORD-2 studies. Lancet HIV 2019;6:e576–87.
[18] Monsalvo M, Vallejo A, Fontecha M, Vivancos MJ, Vizcarra P, Casado JL. CD4/CD8 ratio improvement in HIV-1-infected patients receiving dual antiretroviral treatment. Int J STD AIDS 2019;30:656–62.
[19] Moreno S, Perno CF, Mallon PW, Behrens G, Corbeau P, Routy JP, et al. Two-drug vs. three-drug combinations for HIV-1: do we have enough data to make the switch? HIV Med 2019;20:2–12.
[20] Lima VD, Wang L, Brumme C, Wu L, Montaner JSG, Harrigan PR. Estimation of measurement error in plasma HIV-1 RNA assays near their limit of quantification. PLoS One 2017;12:e0171155.
[21] Lenth RV. Post hoc power: tables and commentary. Department of Statistics and Actuarial Science; 2007. Technical Report No. 378.

[22] Hayati Rezvan P, Lee KJ, Simpson JA. The rise of multiple imputation: a review of the reporting and implementation of the method in medical research data collection, quality, and reporting. BMC Med Res Methodol 2015;15:1–14.
[23] Maggiolo F, Di Filippo E, Valenti D, Serna Ortega P, Callegaro A. NRTI sparing therapy in virologically controlled HIV-1 infected subjects. J Acquir Immune Defic Syndr 2016;72:46–51.
[24] Vallejo A, Molano M, Monsalvo-Hernando M, Hernández-Walias F, Fontecha-Ortega M, Casado J. Switching to dual antiretroviral regimens is associated with improvement or no changes in activation and inflammation markers in virologically suppressed HIV-1-infected patients: the TRILOBITHE pilot study. HIV Med 2019;20:555–60.
[25] Lombardi F, Belmonti S, Quiros-Roldan E, Latini A, Castagna A, D'Ettorre G, et al. Evolution of blood-associated HIV-1 DNA levels after 48 weeks of switching to atazanavir/ritonavir+lamivudine dual therapy versus continuing triple therapy in the randomized AtLaS-M trial. J Antimicrob Chemother 2017;72:2055–9.