WILEY

Frontiers in Flow Cytometry™

24 hour Virtual Event

September 13th, 2023

Frontiers in Flow Cytometry™ is for researchers across the globe looking for an opportunity to share and learn about current developments in flow cytometry. This 24 hour virtual event will feature keynote presentations by industry colleagues, webinars, demos, live networking opportunities and more.

Key topics include:

- Spectral and conventional flow cytometry
- Immunophenotyping and Standardization
- Panel design and optimization
- Cancer Biology and Auto-immune Diseases
- Infectious diseases
- Advances in flow cytometry technology

Register Now

This event is sponsored by **ThermoFisher** SCIENTIFIC

RESEARCH ARTICLE

HIV-DNA decrease during treatment in primary HIV-1 infection with three different drug regimens: Italian Network of Acute HIV Infection (INACTION) clinical trial

Elena Bruzzesi[1] ![ORCID] | Arianna Gabrieli[2] ![ORCID] | Davide Bernasconi[3] ![ORCID] |
Giulia Marchetti[4,5] ![ORCID] | Andrea Calcagno[6] ![ORCID] | Diego Ripamonti[7] ![ORCID] |
Andrea Antinori[8] ![ORCID] | Nicola Squillace[9] ![ORCID] | Antonella Cingolani[10,11] ![ORCID] |
Antonio Muscatello[12] ![ORCID] | Alessandra Bandera[5,12] ![ORCID] | Andrea Gori[5] |
Stefano Rusconi[5,13] ![ORCID] | Silvia Nozza[1,14] ![ORCID] | INACTION Study Group

[1]Vita-Salute San Raffaele University, Milan, Italy

[2]Dipartimento di Scienze Biomediche e Cliniche (DIBIC), Milan, Italy

[3]Bicocca Bioinformatics Biostatistics and Bioimaging Centre - B4 School of Medicine and Surgery, University of Milano-Bicocca, Monza, Italy

[4]Clinic of Infectious Diseases, Department of Health Sciences, ASST Santi Paolo e Carlo, University of Milan, Milan, Italy

[5]School of Medicine and Surgery, University of Milan, Milan, Italy

[6]Unit of Infectious Diseases, Department of Medical Sciences, University of Turin, Turin, Italy

[7]Infectious Disease Unit, ASST Papa Giovanni XXIII, Bergamo, Italy

[8]National Institute for Infectious Diseases Lazzaro Spallanzani IRCCS, Rome, Italy

[9]Infectious Disease Unit, Fondazione IRCCS San Gerardo dei Tintori, Monza, Italy

[10]Istituto Clinica di Malattie Infettive, Università Cattolica del Sacro Cuore, Rome, Italy

[11]UOC Malattie Infettive, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy

Abstract

As the introduction of antiretroviral therapy (ART) during primary HIV-1 infection (PHI) could restrict the establishment of HIV reservoirs, we aimed to assess the effect of three different ART regimens on HIV-DNA load in people living with HIV (PLWH), who started ART in PHI. Randomized, open-label, multicentric study, including subjects in PHI (defined as an incomplete HIV-1 Western blot and detectable plasma HIV-RNA) in the Italian Network of Acute HIV Infection cohort. Participants were randomly assigned (10:10:8) to a fixed-dose combination of tenofovir alafenamide fumarate (TAF) 10 mg plus emtricitabine (FTC) 200 mg, darunavir 800 mg, and cobicistat 150 mg once daily (group A), or TAF 25 mg plus FTC 200 mg, dolutegravir 50 mg once daily (group B), or an intensified four-drug regimen (TAF 10 mg plus FTC 200 mg, dolutegravir 50 mg, darunavir 800 mg, and cobicistat 150 mg once daily) (group C). The primary endpoint was the decrease of HIV-DNA copies/10^6 peripheral blood mononuclear cells (PBMCs) at weeks (W) 12 and 48. Secondary endpoints were increased in CD4+ cells and in CD4+/CD8+ ratio and percentage of PLWH reaching undetectable HIV-RNA. HIV-DNA was quantified by Droplet Digital PCR (Biorad QX100) and normalized to RPP30 reference gene. This study was registered in ClinicalTrials.gov (number NCT04225325). Among 78 participants enrolled, 30 were randomized to group 1, 28 to group 2, and 20 to group 3. At baseline, median CD4+ count was 658/μL (476–790), HIV-RNA 5.37 (4.38, 6.12) $\log_{10}$ copies/mL, without statistical difference in their change among groups at weeks 12 and 48 ($p = 0.432$ and 0.234, respectively). The trial was prematurely discontinued for slow accrual and for

---

[12]Infectious Diseases Unit, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan

[13]SC Malattie Infettive, Ospedale di Legnano, ASST Ovest Milanese, Legnano, Italy

[14]Infectious Diseases Unit, IRCCS Ospedale San Raffaele, Milan, Italy

Correspondence
Silvia Nozza, Via Stamira d'Ancona 20, 20127 Milano, Italy.
Email: nozza.silvia@hsr.it

COVID-19 pandemic-associated restrictions. In the per-protocol analysis, PLWH ($n = 72$) with undetectable viral load was 54.3% at W12 and 86.4% at W48. Interestingly, the CD4/CD8 ratio progressively increased over time, up to normalization in almost half of the cohort by week 48, despite a deflection in group 3; no difference was observed by the Fiebig stage (I–III vs. IV–VI). HIV-DNA decreased from 4.46 (4.08, 4.81) $\log_{10}$ copies/10^6 PBMCs to 4.22 (3.79, 4.49) at week 12, and 3.87 (3.46, 4.34) at week 48, without difference among groups. At multivariable analysis, HIV-DNA delta at W48 was associated only with the increase of CD4+ count by 100 cells/mm^3 but not with the Fiebig stage, the CD4+/CD8+ ratio, and treatment arm, despite a higher decrease in group 3. Six adverse events were recorded during our study, which did not cause any withdrawal from the study. We observed a decrease in HIV-DNA from baseline to W48 in PLWH treated during PHI, associated with an increase in CD4+ count, unrelated to the treatment arm.

KEYWORDS
antiretroviral therapy, HIV, HIV-DNA, primary HIV infection

1 | INTRODUCTION

Primary HIV-1 infection (PHI) is a peculiar phase that encompasses the first months after transmission, characterized by the peak of viral load in blood and genital secretions with the highest HIV infectiousness and by the activation of the immune system resulting in the development of detectable HIV antibodies and to immune exhaustion. Fiebig staging provides a temporal description of the above-mentioned events.[1]

Although antiretroviral therapy (ART) efficiently inhibits viral replication, it does not eradicate the HIV reservoir, being the major barrier to a functional cure. HIV-DNA is a marker of the HIV reservoir which predicts HIV progression[2]; in acute infection HIV-DNA reaches its peak and its set point very rapidly, in a similar fashion as HIV-RNA viremia.[3] Interestingly, initiating ART early in PHI limits the size of the reservoir, particularly if started in Fiebig 3 or less,[4–6] and provides an enhanced immune control.[7] Intriguingly, HIV-DNA is markedly reduced when ART is introduced in the first 2 weeks, confirming the association between longer time to ART initiation and reservoir size.[8] Moreover, very early initiation of the ART was described to grant long-term virological remission in only few cases.[9] Nevertheless, as the rapid introduction of ART limits the HIV reservoir, T-cell exhaustion, and genetic diversity,[10] people living with HIV (PLWH) treated in PHI could be interesting candidates for curative interventions. Thus, it is crucial to investigate the best strategies to manage ART in this particular phase of HIV infection. Data from previous studies suggest that reservoir size is not affected by treatment intensification with raltegravir and maraviroc, as compared with standard ART[11,12] and by type of regimen when comparing dolutegravir-based regimen with darunavir-based regimen.[13]

In this study, the primary hypothesis was that blocking HIV replication and in particular integration within the host genome in PLWH starting therapy during PHI could result in a reduction of reservoir size, determined as HIV-DNA. In particular, the primary aim of the P25-INACTION trial, an Italian randomized prospective study, enrolling PLWH who started ART during PHI with emtricitabine (FTC)/tenofovir alafenamide fumarate (TAF) as backbone plus darunavir/cobicistat or dolutegravir or an intensified regimen including all four active compounds, was to compare the dynamics of HIV-DNA at weeks 12 and 48.

2 | METHODS

2.1 | Study design and participants

The P25-INACTION trial was a randomized, parallel-group, open-label, multicentric study conducted in nine Italian university hospitals provided with dedicated HIV services for outpatients, included in the Italian Network of Acute HIV Infection (INACTION) national network. Patients were included from April–May 2018 to March 2020. Inclusion criteria were adult patients diagnosed during the early PHI phase, symptomatic or not, with HIV antibodies at the third-generation (or further) enzyme-linked immunosorbent assay and Western blot indicative of HIV infection in Fiebig stages I–VI[1] and able to sign the informed consent.

The main exclusion criteria were pregnant or breastfeeding women, will of pregnancy, active opportunistic infection or malignancy, positivity for Hepatitis B at screening (HBsAg+) or anticipated need for Hepatitis C virus therapy during the study, alanine aminotransferase (ALT) five times the upper limit of normal (ULN) or ALT 3 × ULN and bilirubin 1.5 × ULN (with >35% direct bilirubin) or severe hepatic impairment (Class C) as determined by Child-Pugh classification, creatinine clearance of <70 mL/min via Cockroft-Gault method and drug–drug interaction.

Participants were asked to be enrolled in several substudies encompassing invasive procedures, such as lumbar puncture, lymph node biopsies, and colonoscopy, upon signing dedicated informed consent. The study was approved by the institutional Ethics Committee of San Raffaele Hospital (coordinating center). All participants gave the written informed consent.

This study is registered under the number NCT04225325 on ClinicalTrials.gov.

2.2 | Randomization and blinding

Randomization was performed in a ratio 10:10:8 through a computer-generated allocation sequence, stratified according to Fiebig stages (I–III, IV–V, and VI groups), in balanced blocks. Patients and care providers were not blind to treatment allocation.

2.3 | Procedures

Eligible participants were randomly assigned (10:10:8) to a fixed-dose combination of TAF 10 mg plus FTC 200 mg, darunavir 800 mg, and cobicistat 150 mg once daily (group A), or TAF 25 mg plus FTC 200 mg, dolutegravir 50 mg once daily (group B), or an enhanced four-drug regimen (TAF 10 mg plus FTC 200 mg, dolutegravir 50 mg, darunavir 800 mg, and cobicistat 150 mg once daily) (group C). Participants were enrolled in the centers by medical doctors; following randomization, treatment were prescribed and dispensed by the hospital pharmacy.

Clinical examinations and laboratory tests were performed at baseline and at baseline, week 2 (W2), W4, W8, W12, W24, W36, and W48. Plasma HIV-1-RNA levels, CD4+ and CD8+T-cell counts, blood cell counts, and blood chemistry values were measured each time.

Total HIV-DNA was extracted from the peripheral blood mononuclear cell (PBMC) previously isolated, using *Qiamp DNA Mini Kit*, following the manufacturer's protocol. Extracted DNA was digested using the restriction enzyme MSCII (Thermo Fischer Scientific) at 37°C for 1 h. Primer/probe set to conserved regions of HIV-gag and an RPP30 (RNAse P) primer/probe set were used for HIV-DNA quantification. Samples were diluted 10-fold for the RPP30 assay. Total HIV-DNA was measured in duplicate on droplet digital polymerase chain reaction (ddPCR) with the QX100 Droplet Digital PCR platform (Biorad). The ddPCR reaction mix consisted of 10 μL 2XddPCR super mix for probes (Biorad), 750 nM primers, 250 nM probes, and 5 μL of digested DNA. ddPCR amplification reactions consisted of initial denaturation at 95°C for 10 min, followed by 39 cycles of 94°C for 30 s denaturation, 55°C for 1 min annealing, and 98°C for 10 min elongation. Droplets were read by the QX100 droplet reader and the data analyzed using the QuantaSoft analysis software (Biorad Quanta Soft v 1.7). Results are reported as copies/10^6 PBMCs, and the quantities of total HIV-DNA were normalized to a reference gene RPP30 measured by ddPCR.

The study was conducted in conformance with Good Clinical Practices.

2.4 | Outcomes

The aim of the study was to compare the virological efficacy of an enhanced four-drug antiretroviral regimen against a standard regimen (chosen as the best one between an integrase-based or a protease inhibitor-based three drugs regimen) in people with PHI. The primary endpoint was the change in HIV-1-DNA/10^6 PBMCs at W48 among the three groups. The prespecified secondary endpoints included the percentage of subjects with HIV-1 RNA < 50 copies/mL at weeks 24 and 48, time to achieve undetectable viral load (HIV-1 RNA < 50 copies/mL), change in HIV-DNA at weeks 12 and 48; change from baseline in CD4+T-cell count, percentage of CD4+T-cell, CD8++T-cell count, percentage of CD8+T-cell, the CD4/CD8 ratio at week 48, percentage of patients with CD4/CD8 > 1 at week 48.

2.5 | Statistical analysis

For sample size calculation, the expected results on the primary endpoint (HIV-DNA) were based on data from the OPTIPRIM-ANRS 147 study.[11] The planned sample size was 37 patients per group to demonstrate a difference of 0.4 $\log_{10}$ copies/10^6 PBMC between arms B and A with a power of 80%, considering a standard deviation of 0.6 in both groups, a type I error rate of 5% and a two-sided *T* test. It was then calculated that by including 30 patients in arm C, a power of 85% is achieved to demonstrate a difference of 0.6 $\log_{10}$ copies/10^6 PBMC between arm C and arm A considering a standard deviation of 0.8 in arm C, a type I error rate of 5% and a two-sided *T* test. Accounting for a possible 5% drop-outs in all arms, we increased the final sample sizes to 40, 40, and 32 for arms A, B, and C, respectively.

Demographics and immunovirological characteristics were described using median and interquartile range (IQR) for continuous variables and absolute frequency and percentage for categorical variables. Comparison among treatment groups was performed using the Kruskal–Wallis test for continuous variables and the Chi-square test for categorical variables.

Change in time from baseline to weeks 4, 8, 12, 24, 36, and 48 of HIV-RNA and CD4 cell count was also compared among treatment groups using repeated measures analysis of variance (ANOVA). Analysis of the primary endpoint was conducted for the per-protocol population, defined as all randomized patients who initiated treatment and whose HIV-DNA level was measured over time. The association between treatment group and change from baseline to week 12, and separately for change to week 48, was assessed by one-way ANOVA. Finally, to adjust the association between treatment and HIV-DNA change (both at weeks 12 and 48) for the impact of possible residual confounding not addressed by randomization, we performed a multivariable linear regression including as covariates: age (years), Fiebig stage (IV–VI vs. I–III), CD4+ cells/mm^3, CD4+/CD8+ ratio and HIV-RNA $\log_{10}$(copies/mL).

All statistical analyses were performed using the R software (version 4.3.0).

3 | RESULTS

Between May 2018 and March 2020, 78 individuals were enrolled, 30 were randomly assigned at group A, 28 at group B, and 20 at group C (Figure 1). The trial was prematurely discontinued for slow accrual and for COVID-19 pandemic-associated restrictions.

At baseline, the median CD4+ cells count was 540 (IQR = 394–653) cells/mm^3, CD4+ cells percentage was 22.1 (14.6–28), and the CD4/CD8 ratio was 0.39 (0.23–0.58). HIV-RNA was 5.66 (4.62–6.5) $\log_{10}$ copies/mL and HIV-DNA was 4.46 (4.08–4.81) $\log_{10}$ copies/10^6 PBMCs.

Immunovirological and demographic baseline characteristics were comparable in all groups (Table 1); all the participants were infected with a wild-type virus.

At week 12, median HIV-DNA decreased from 4.46 (4.08–4.80) to 4.22 (3.79–4.49) $\log_{10}$ copies/10^6 PBMCs, without difference between groups. The mean change was −0.329 (0.525) in group A, +0.016 (1.192) increase in group B, and −0.355 (0.381) in group C. At week 48, HIV-DNA decreased significantly from 4.46 (IQR = 4.08–4.80) to 3.85 (3.49–4.37)
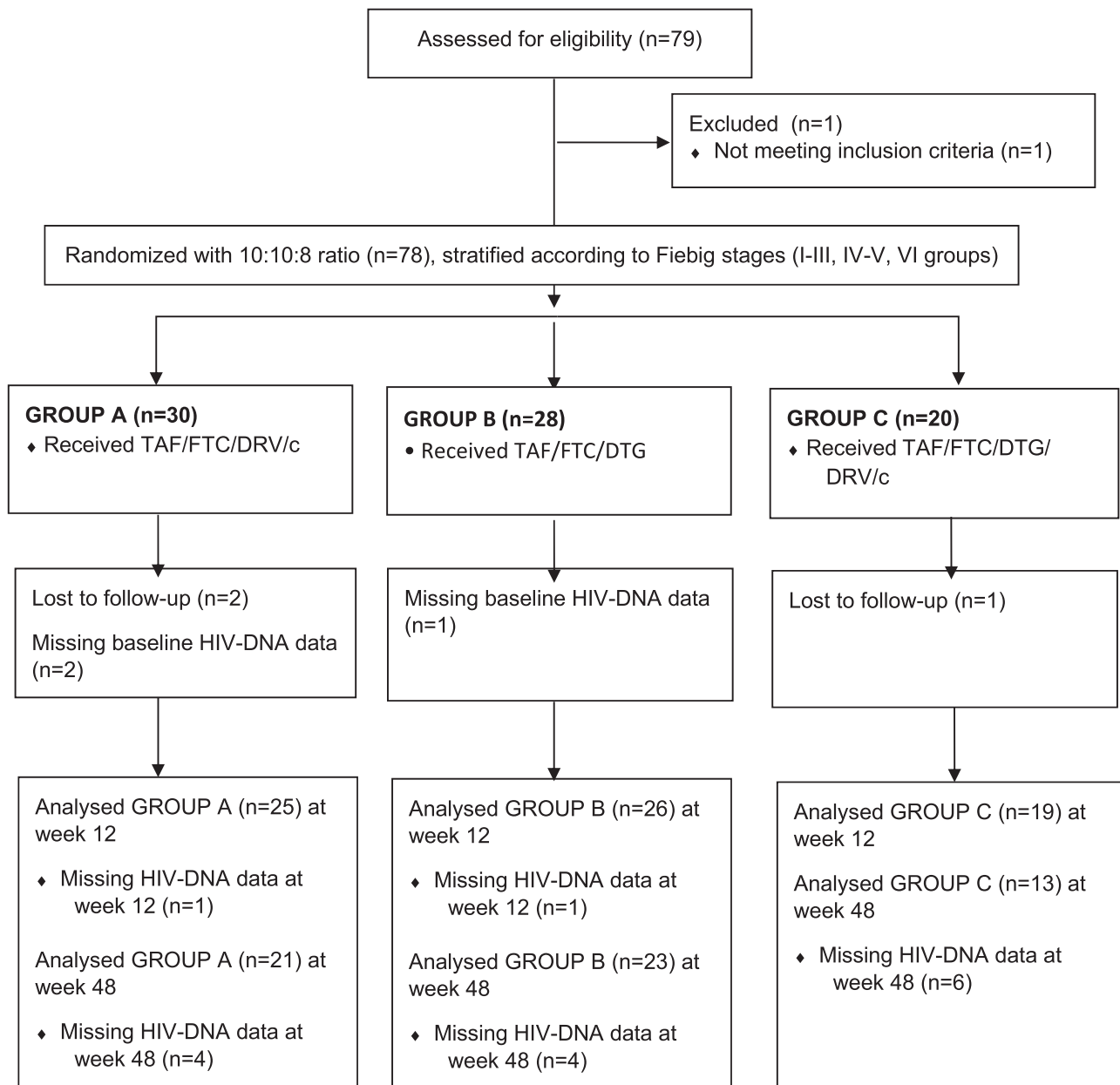

FIGURE 1 Enrollment and randomization. DRV/c, darunavir/cobicistat; DTG, dolutegravir; FTC, emtricitabine; TAF, tenofovir alafenamide fumarate.

TABLE 1 Baseline characteristics of enrolled PLWH by treatment arm.

Factors	Level/unit	Randomly assigned treatment arm			
		TAF/FTC + DRV/c N = 30	TAF/FTC + DTG N = 28	TAF/FTC + DRV/c + DTG N = 20	p*
Fiebig (%)	1	1 (3.3)	2 (7.4)	1 (5.6)	0.262
	2	8 (26.7)	4 (14.8)	4 (22.2)	
	3	1 (3.3)	3 (11.1)	0 (0.0)	
	4	6 (20.0)	0 (0.0)	4 (22.2)	
	5	11 (36.7)	13 (48.1)	5 (27.8)	
	6	3 (10.0)	5 (18.5)	4 (22.2)	
Gender (%)	F	3 (10.0)	0 (0.0)	1 (5.0)	0.319
	M	26 (86.7)	28 (100.0)	19 (95.0)	
	Transgender	1 (3.3)	0 (0.0)	0 (0.0)	
Age (median [IQR])		34.99 [29.17, 43.50]	32.76 [28.08, 41.16]	33.16 [26.66, 46.54]	0.832
Days from HIV diagnosis to ART start (median [IQR])		12 [3, 34]	12 [6, 23]	13 [7, 18]	0.990
Risk factor (%)	Bisexual	1 (4.3)	0 (0.0)	0 (0.0)	0.741
	Hetero	1 (4.3)	2 (7.7)	1 (5.6)	
	Homo	5 (21.7)	9 (34.6)	4 (22.2)	
	Transgender	16 (69.6)	15 (57.7)	13 (72.2)	
HCV (%)	No	27 (96.4)	25 (96.2)	19 (100.0)	0.695
	Yes	1 (3.6)	1 (3.8)	0 (0.0)	
CD4 count (median [IQR])	Cells/mm^3	547.00 [397.50, 669.50]	552.00 [394.00, 653.00]	474.00 [430.00, 584.00]	0.875
CD4 percentage (median [IQR])	%	23.10 [18.30, 27.65]	18.00 [11.30, 29.00]	19.00 [15.00, 27.60]	0.477
CD4/CD8 (median [IQR])		0.47 [0.30, 0.59]	0.35 [0.17, 0.58]	0.33 [0.23, 0.50]	0.499
HIV-RNA (median [IQR])	log$_{10}$(copies/mL)	5.17 [4.40, 6.02]	5.43 [4.27, 6.04]	5.75 [4.83, 6.22]	0.708
HIV-DNA (median [IQR])	log$_{10}$(copies/10^6 PBMCs)	4.39 [4.08, 4.73]	4.53 [4.22, 4.83]	4.45 [4.11, 4.79]	0.600

Note: Comparison among treatment groups was performed using the Kruskal–Wallis test for continuous variables and the Chi-square test for categorical variables.

Abbreviations: DRV/c, darunavir/cobicistat; DTG, dolutegravir; FTC, emtricitabine; HCV, hepatitis C virus; IQR, interquartile range; PBMCs, peripheral blood mononuclear cells; TAF, tenofovir alafenamide fumarate.

log$_{10}$ copies/10^6 PBMCs, without difference between groups. The mean change was −0.912 (SD = 1.553) in group A, −0.267 (1.598) in group B, and −0.988 (1.237) in group C, with a larger, still not significant, average decrease of HIV-DNA load in treatment groups including DRV (groups A and C) compared with group B (Figure 2). Of note, there were two subjects with the advanced Fiebig stage (stage V) that had an HIV-DNA level of zero at baseline on repeated measures and positive values at weeks 12 and 48, with a delta of around 4 log. In depth, comparing the very early Fiebig stages I and II with subsequent stages III and IV and late stages V and VI, we observed no statistical difference for HIV-DNA at baseline (p = 0.739). Interestingly, a trend of higher decrease of HIV-DNA was observed for the subjects starting treatment in

stages I and II, both at week 12 (p = 0.236) and, even steeper, at week 48 (p = 0.068) (Supporting Information Table 1). However, HIV-DNA at the three time points was not statistically different among treatment arms, both for Fiebig stages I and II (Supporting Information Table 2) and for Fiebig stages III–VI (Supporting Information Table 3).

Multivariable linear regression analysis performed on the main endpoint at weeks 12 and 48 considered as covariates treatment arm (A vs. C, B vs. C), age (per 10 years), Fiebig stage (IV–VI vs. I–III), CD4+ cells count (per 100 cells/mm^3), the CD4/CD8 ratio (per unit), and HIV-RNA (per log$_{10}$ copies/mL). Factors significantly associated with HIV-DNA decrease were CD4+ cells count and the CD4/CD8 ratio (Table 2); there was no difference
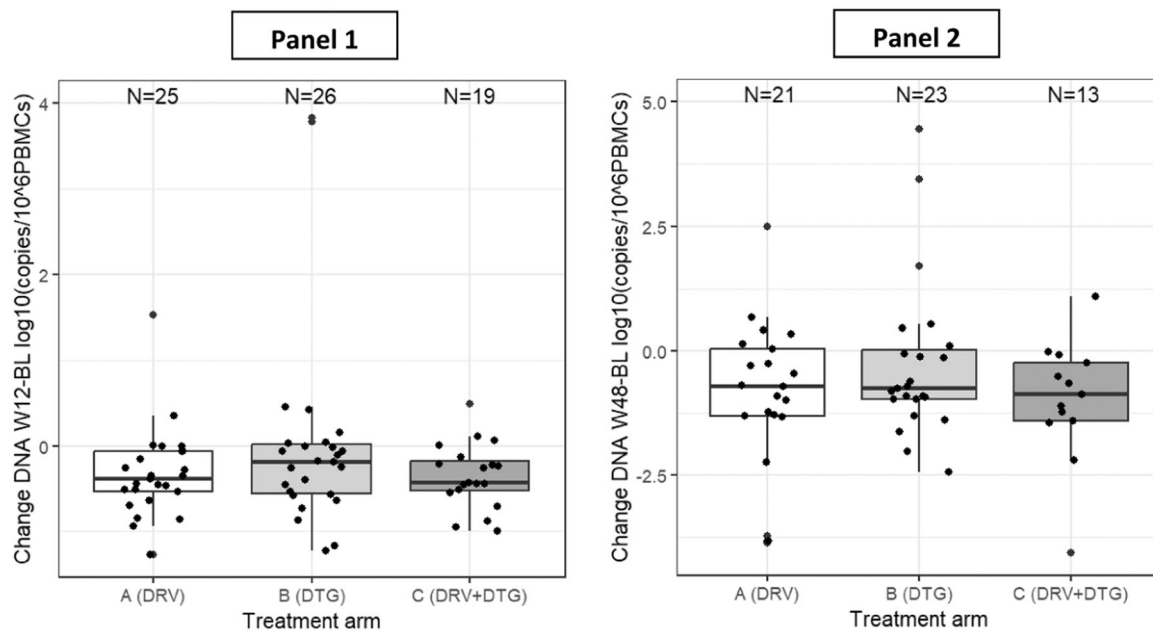
FIGURE 2 Change in HIV-DNA from baseline to W12 (Panel 1) and from baseline to W48 (Panel 2). DRV, darunavir; DTG, dolutegravir; PBMCs, peripheral blood mononuclear cells.

TABLE 2 Multivariable linear regression analysis investigating factors associated with HIV-DNA change from baseline to 12 and 48 weeks.

Variables	Change at week 12 (N = 62)		Change at week 48 (N = 52)	
	Mean difference (95% CI)	p Value	Mean difference (95% CI)	p Value
(Intercept)	0.181 (−1.818; 2.179)	0.857	0.088 (−4.207; 4.383)	0.967
Treatment arm A versus C	0.146 (−0.406; 0.698)	0.598	0.149 (−0.991; 1.289)	0.794
Treatment arm B versus C	0.473 (−0.079; 1.025)	0.091	0.952 (−0.185; 2.09)	0.099
Age, per 10 years	−0.091 (−0.296; 0.114)	0.379	0.025 (−0.382; 0.433)	0.901
Fiebig IV–VI versus I–III	0.364 (−0.184; 0.912)	0.188	0.551 (−0.513; 1.616)	0.302
CD4+ per 100 cells/mm^3	0.082 (−0.010; 0.174)	0.079	0.207 (0.041; 0.373)	0.015
CD4+/CD8+ per unit	−1.226 (−2.237; −0.216)	0.018	−1.956 (−3.976; 0.063)	0.057
HIV-RNA per $\log_{10}$(cp/mL)	−0.082 (−0.309; 0.146)	0.475	−0.358 (−0.822; 0.105)	0.127

Abbreviation: CI, confidence interval.

between treatment groups, but there was a trend of greater HIV-DNA decline in group C. Table 2 reports the results of multivariable analysis at weeks 12 and 48.

Plasma HIV-RNA decreased more rapidly in groups B and C, which contained DTG. Figure 3 shows the percentage of individuals with HIV-RNA < 50 copies/mL at each time point. The percentage of undetectable viral load was higher in groups B and C until week 24 and reached similar values in group A at weeks 36 and 48. A continuous decrease in HIV-RNA levels was observed, with a significant difference between treatment groups on HIV-RNA change in time (repeated measures ANOVA $p < 0.001$, Figure 4). No participants discontinued the treatment due to virological failure.

CD4+T-lymphocytes increased during treatment in all groups (Figure 5). There was no treatment effect on CD4 change in time (repeated measures ANOVA $p = 0.644$). The percentage of participants reaching a CD4+/CD8+ ratio >1 at week 4 was higher in groups B (24%) and C (26.3%) than in group A (12%); the differences between groups were not observed at weeks 36 and 48 (Figure 6). We observed a high percentage of individuals reaching CD4/CD8 > 1 at weeks 4 and 8 according to the Fiebig stage; people treated during stages I–III reached ratio > 1 at week 4 (30% vs. 16.3% stages IV–VI) and week 8 (47, 6% vs. 16.7% stages IV–VI).

Among randomized patients, 34 (43.6%) experienced adverse events: 1 patient had six events, 5 had four events, 6 had
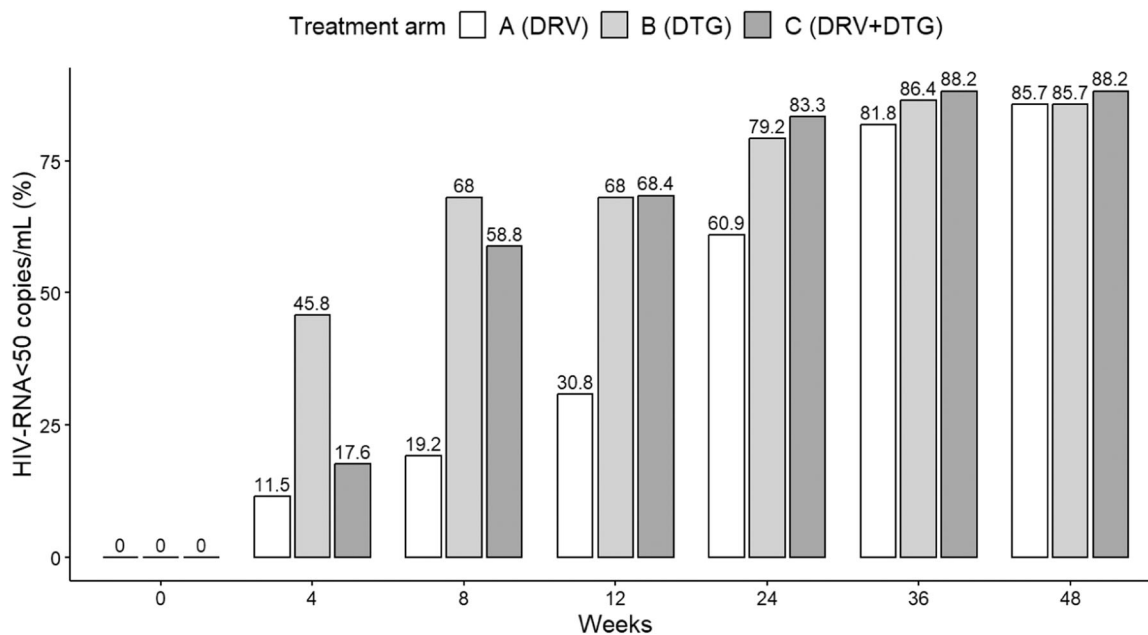
FIGURE 3 Percentage of participants with HIV-RNA < 50 copies/mL over time. DRV, darunavir; DTG, dolutegravir.
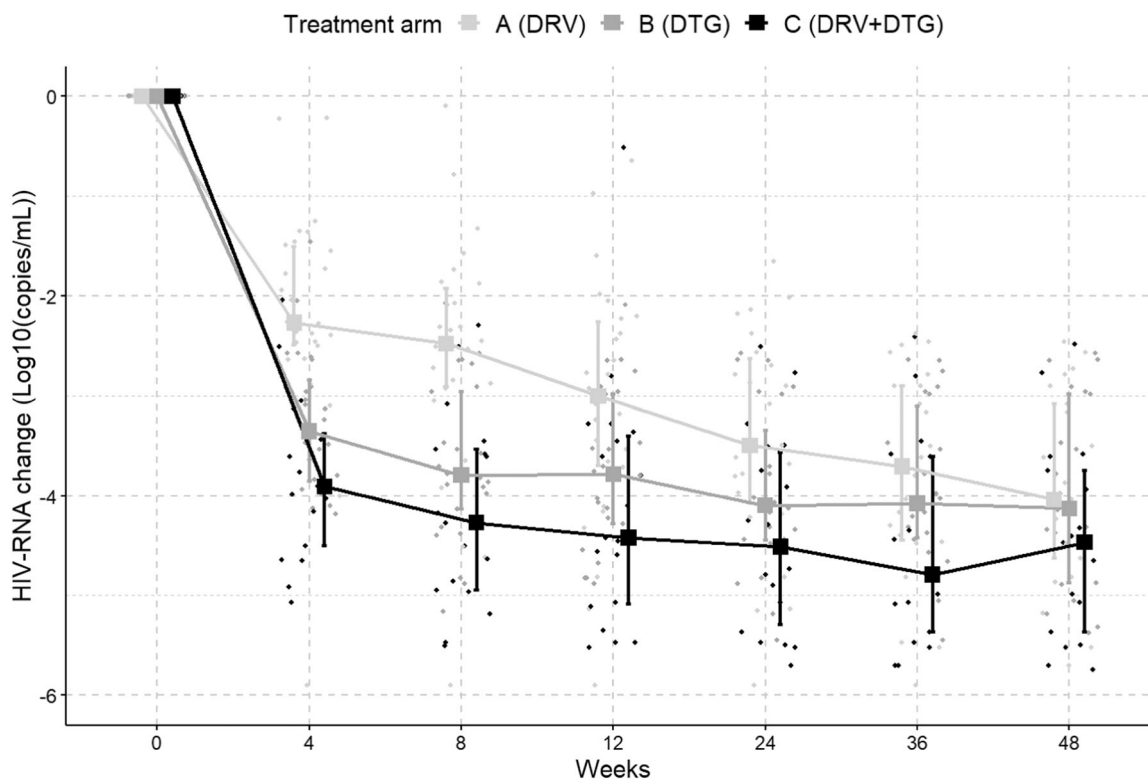

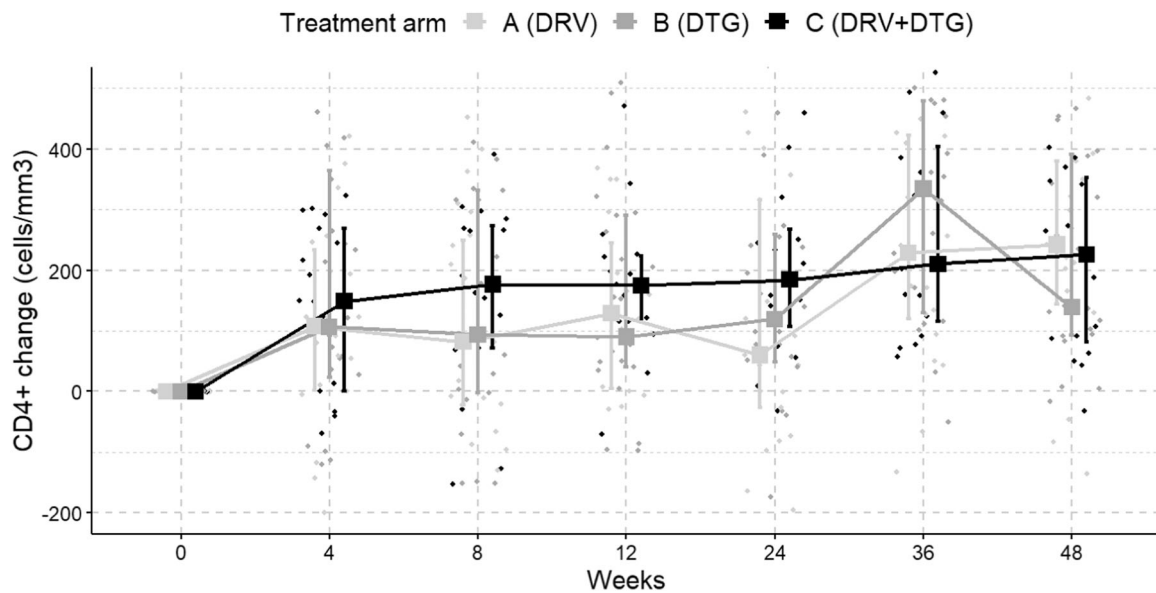**FIGURE 4** HIV-RNA changes over time. Squares show median values, and bars the interquartile range. DRV, darunavir; DTG, dolutegravir.

three events, 9 had two events, and 13 had one event. Six patients (7.6%) had a serious adverse event (one had bacterial cellulitis, one had visual hallucination, one was hospitalized for aortic valve replacement, one had pneumonia from *Pneumocystis jiroveci*, one

had invasive bronchopulmonary aspergillosis and pulmonary pneumocystosis, and one had worsening headache post-lumbar puncture).

Three participants discontinued treatment due to a serious adverse event (one for visual hallucination, one was hospitalized for aortic

FIGURE 5 CD4+ cells count changes over time. Squares represent median values, and bars the interquartile range. DRV, darunavir; DTG, dolutegravir.
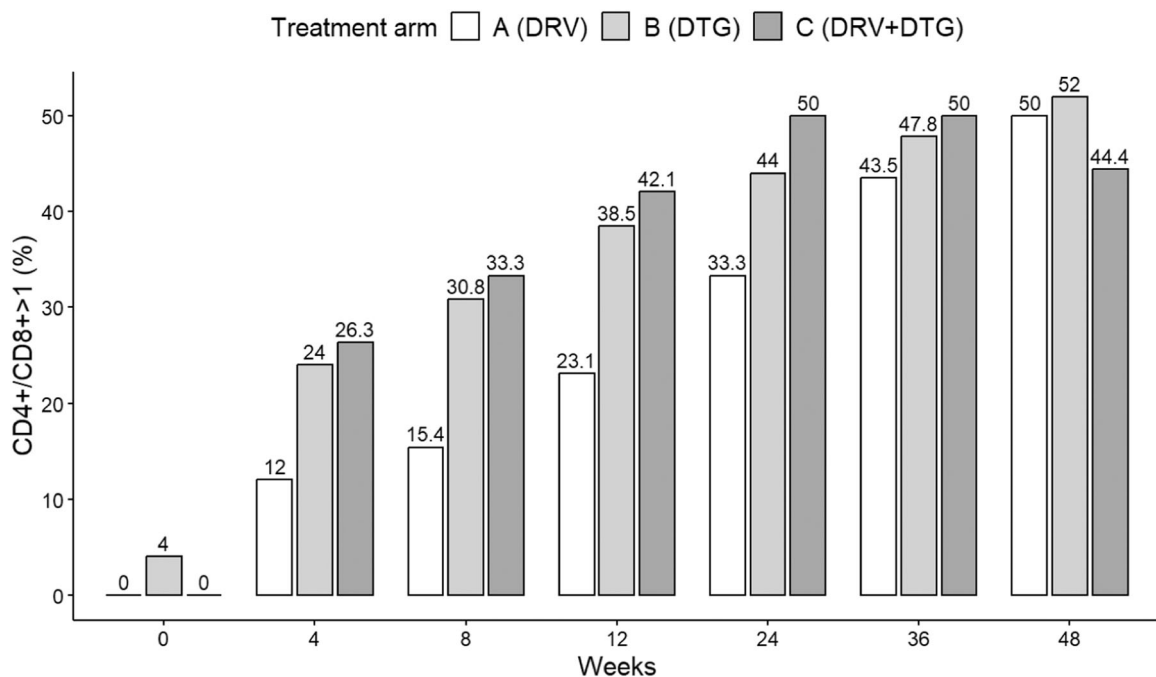

FIGURE 6 Percentage of enrolled PLWH with CD4/CD8 > 1 over time. DRV, darunavir; DTG, dolutegravir; PLWH, people living with HIV.

valve replacement) or an adverse event (one for anxiety–depression symptoms).

4 | DISCUSSION

The P25-INACTION trial is an Italian randomized prospective study which enrolled PLWHIV with PHI. Our hypothesis was that dolutegravir-based ART, initiated during PHI, could more rapidly suppress viral replication and reduce the HIV-reservoir size, after 12 and 48 weeks of treatment, as compared with a PI-based regimen.

HIV reservoir size was measured by total HIV-DNA; although total HIV-DNA does not distinguish between defective and intact provirus, as the intact proviral DNA assay, or the replication-competent virus, as the quantitative viral outgrowth assay, it has clinical significance as it correlates with disease progression,[14,15] of the time of viral rebound after treatment interruption,[16] of the risk of virological failure after treatment de-escalation[17] and of sustained virological response.[18]

HIV-DNA decreased during treatment without differences between treatment arms; however, we confirmed that prompt ART initiation could have a higher impact on the decay of HIV-DNA after 48 weeks, despite similar baseline reservoir size and regardless treatment with PI and/or INSTI. As the HIV reservoir is established in PHI,[4] treatment represents a major opportunity to reduce HIV reservoirs and achieve optimal immune reconstitution. In a prospective, single-center cohort study of 370 PLWHIV, HIV-DNA levels were lower in the group treated during PHI than in the group treated during chronic HIV infection.[19] Another study showed that 31% of individuals with HIV infection did not show a negative HIV-DNA slope 4–7 years from the initiation of ART.[20] Moreover, the results are in accordance with previous studies assessing the decay of HIV-DNA in people starting ART with more than three drugs during PHI.[11,12]

In an observational Italian study, both an intensified four-drug regimen based on raltegravir and boosted DRV and a DTG-based triple ART, showed a similar chance of reaching low-level HIV-DNA.[21]

Another recently published Phase 3 trial, the OPTIPRIM2-ANRS 169, compared HIV-DNA decrease in two different ART regimens containing dolutegravir and darunavir/cobicistat: the reduction was similar in both groups, highlighting that reservoir reduction is not linked to treatment but to the early ART initiation.[13] In P25-INACTION study we observed a continuous decay of HIV-DNA until W48; the PRIMO cohort study group also modeled the decrease of HIV-DNA during effective treatment and recommended early ART in PHI.[22]

Viral replication decreased more in groups treated with DTG-containing regimens (groups B and C) than in triple DRV/c-containing regimen (group A), confirming the more rapid viral load suppression in naïve PLWH initiating an INSTI-based regimen[23]: more than 60% of participants randomized in groups B and C achieved undetectable viral load at W12. Of note, the decay was slower in the intensification group, particularly at week 4; however, it could be due to the higher viremia at baseline. Current guidelines recommend the use of the second-generation INSTI-based regimens in PLWHIV naïve to antiretroviral treatment. These recommendations are extended to PHI treatment, even if data on INSTI use in the context of PHI have been accumulating only in more recent years.[11,13,24] This is pivotal because the main risk of HIV transmission remains sexual contact and high HIV-RNA load is associated with an increased risk of sexual transmission during PHI.[25] OPTIPRIM2-ANRS 169 trial showed that a DTG-based regimen reduces HIV-RNA as well as HIV-DNA present in the male genital compartment, efficiently reducing the high risk of transmission during PHI.[26] Our study confirmed the high genetic barrier of DTG- and DRV/c-based regimens, with no virological failure.

The immune recovery was comparable between groups. However, the probability of achieving a CD4/CD8 ratio ≥1 within the first 12 weeks of ART initiation was higher in groups B and C, treated with dolutegravir. A rapid normalization of the CD4/CD8 ratio was already observed in the setting of PHI.[27] The correlation between the probability of early CD4/CD8 > 1 and the Fiebig stage suggests that a preserved immunological condition at the time of ART initiation plays a key role in immune restoration.

Finally, our study confirmed the tolerability of ART in the PHI setting, with only 3 subjects who discontinued therapy due to an adverse event.

This study has some limitations, including the limited size of the population that did not achieve the expectations. The principal reasons are the decrease in observation of PHI in Italy linked to the effectiveness of treatment as prevention (TaSP) and of pre-exposure prophylaxis (PrEP). We also decided to stop the enrollment during COVID-19 pandemic in 2020 due to our dramatic outpatient situation. Other limitations are the use of HIV-DNA as the only measurement to estimate the size of the reservoir, half of the enrolled subjects were in Fiebig stages 5 and 6, and the lack of information about patient adherence.

In conclusion, P25-INACTION trial demonstrated a decrease of HIV-DNA during 48 weeks of treatment during PHI, without correlation to different ART regimens. DTG-based regimens achieved undetectable viral load in most individuals at W12 and this is crucial for preventing HIV sexual transmission.

AUTHOR CONTRIBUTIONS

Elena Bruzzesi wrote the article and followed the participants. Arianna Gabrieli wrote the article and performed HIV-DNA analysis. Silvia Nozza and Stefano Rusconi conceived the study, wrote the protocol, and coordinated the INACTION Study Group in Italy. Davide Bernasconi performed the statistical analysis. Giulia Marchetti, Andrea Calcagno, Diego Ripamonti, Andrea Antinori, Nicola Squillace, Antonella Cingolani, Antonio Muscatello, and Alessandra Bandera followed participants, coordinated activity in their clinical centers.

INACTION STUDY GROUP

Infectious Diseases Unit, IRCCS Ospedale San Raffaele, Milan, Italy: Elena Bruzzesi, Antonella Castagna, Liviana Della Torre, Silvia Nozza, Mariarita Parisi, Angelo Roberto Raccagni, Giuseppe Tambussi.

Dipartimento di Scienze Biomediche e Cliniche (DIBIC), Milan, Italy: Cecilia Bonazzetti, Tiziana Formenti, Arianna Gabrieli, Andrea Giacomelli, Alessia Lai, Stefano Rusconi.

Clinic of Infectious Diseases, Department of Health Sciences, ASST Santi Paolo e Carlo, University of Milan, Milan, Italy: Francesca Bai, Valeria Bono, Camilla Falcinella, Giulia Maria Marchetti, Camilla Tincati.

Unit of Infectious Diseases, Department of Medical Sciences, University of Turin, Turin, Italy: Stefano Bonora, Andrea Calcagno, Jessica Cusato, Antonio D'Avolio, Ilaria De Benedetto, Amedeo De Nicolò, Giovanni Di Perri, Micol Ferrara, Mattia Trunfio.

Infectious Disease Unit, ASST Papa Giovanni XXIII, Bergamo, Italy: Diego Ripamonti, Franco Maggiolo, Daniela Valenti.

National Institute for Infectious Diseases Lazzaro Spallanzani IRCCS, Rome, Italy: Andrea Antinori, Carmela Pinnetti.

Infectious Disease Unit, Fondazione IRCCS San Gerardo dei Tintori, Monza, Italy: Paolo Bonfanti, Nicola Squillace.

Istituto Clinica di Malattie Infettive, Università Cattolica del Sacro Cuore, Rome, Italy: Roberto Cauda, Antonella Cingolani, Simona Di Giambenedetto.

Infectious Diseases Unit, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy: Alessandra Bandera, Giorgio Bozzi, Valentina Ferroni, Andrea Gori, Antonio Muscatello, Valeria Pastore.

Azienda ospedaliero-universitaria di Modena, Modena, Italy: Vanni Borghi, Federica Carli, Cristina Mussini.

Ospedale di Catania, Catania, Italy: Benedetto Maurizio Celesia.

Azienda Ospedaliera "Pugliese Ciaccio" di Catanzaro, Catanzaro, Italy: Lucio Cosco, Carlo Torti.

Ospedale Umberto I Roma, Rome, Italy: Gabriella D'Ettorre.

IRCCS AOU San Martino-IST di Genova, Genova, Italy: Antonio Di Biagio.

University of Brescia School of Medicine and Brescia Spedali Civili Hospital, Brescia, Italy: Emanuele Foca, Eugenia Quiros-Roland.

Presidio Ospedaliero Umberto I di Siracusa, Siracusa, Italy: Antonina Franco.

Fondazione IRCCS policlinico San Matteo, Pavia, Italy: Massimiliano Fabbiani, Roberto Gulminetti.

Azienda Ospedaliero-Universitaria di Cagliari, Cagliari, Italy: Marco Campus, Marzia Garau, Sandro Piga.

Department of Medical, Surgical and Experimental Sciences, University of Sassari, Sassari, Italy: Giordano Madeddu.

CONFLICT OF INTEREST STATEMENT

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ETHICS STATEMENT

The study was approved by the Ethical Committee, Ospedale San Raffaele, Milan, Italy. All participants signed informed consent. This study is registered under the number NCT04225325 on ClinicalTrials.gov.

ORCID

Elena Bruzzesi http://orcid.org/0000-0002-3601-7686
Arianna Gabrieli http://orcid.org/0000-0002-2657-8025
Davide Bernasconi http://orcid.org/0000-0001-8771-5428
Giulia Marchetti http://orcid.org/0000-0001-8860-8632
Andrea Calcagno http://orcid.org/0000-0003-2534-8815
Diego Ripamonti http://orcid.org/0000-0001-6702-6668
Andrea Antinori http://orcid.org/0000-0003-2121-4684
Nicola Squillace http://orcid.org/0000-0002-7608-6045
Antonella Cingolani http://orcid.org/0000-0002-3793-2755
Antonio Muscatello http://orcid.org/0000-0002-2428-6432
Alessandra Bandera http://orcid.org/0000-0001-9440-4601
Stefano Rusconi https://orcid.org/0000-0002-0375-9990
Silvia Nozza https://orcid.org/0000-0002-2673-2731

REFERENCES

1. Fiebig EW, Wright DJ, Rawal BD, et al. Dynamics of HIV viremia and antibody seroconversion in plasma donors: implications for diagnosis and staging of primary HIV infection. *AIDS*. 2003;17(13):1871-1879. doi:10.1097/00002030-200309050-00005
2. Tierney C, Lathey JL, Christopherson C, et al. Prognostic value of baseline human immunodeficiency virus type 1 DNA measurement for disease progression in patients receiving nucleoside therapy. *J Infect Dis*. 2003;187(1):144-148. doi:10.1086/3458709
3. Ananworanich J, Chomont N, Eller LA, et al. HIV DNA set point is rapidly established in acute HIV infection and dramatically reduced by early ART. *EBioMedicine*. 2016;11:68-72.
4. Strain MC, Little SJ, Daar ES, et al. Effect of treatment, during primary infection, on establishment and clearance of cellular reservoirs of HIV-1. *J Infect Dis*. 2005;191(9):1410-1418. Epub March 29, 2005. doi:10.1086/428777
5. Pinzone MR, Graf E, Lynch L, et al. Monitoring integration over time supports a role for cytotoxic T lymphocytes and ongoing replication as determinants of reservoir size. *J Virol*. 2016;90: 10436-10445.
6. Leyre L, Kroon E, Vandergeeten C, et al. Abundant HIV-infected cells in blood and tissues are rapidly cleared upon ART initiation during acute HIV infection. *Sci Transl Med*. 2020;12:eaav3491.
7. Ndhlovu ZM, Kazer SW, Nkosi T, et al. Augmentation of HIV-specific T cell function by immediate treatment of hyperacute HIV-1 infection. *Sci Transl Med*. 2019;11:eaau0528.
8. Shelton EM, Reeves DB, Bender Ignacio RA. Initiation of antiretroviral therapy during primary HIV infection: effects on the latent HIV reservoir, including on analytic treatment interruptions. *AIDS Rev*. 2020;23(1):28-39. doi:10.24875/AIDSRev.20000001
9. Sáez-Cirión A, Bacchus C, Hocqueloux L, et al. Post-treatment HIV-1 controllers with a long-term virological remission after the interruption of early initiated antiretroviral therapy ANRS VISCONTI study. *PLoS Pathog*. 2013;9(3):e1003211.
10. Bacchus C, Cheret A, Avettand-Fenoël V, et al. A single HIV-1 cluster and a skewed immune homeostasis drive the early spread of HIV among resting CD4+ cell subsets within one month post-infection. *PLoS ONE*. 2013;8(5):e64219.
11. Chéret A, Nembot G, Mélard A, et al. OPTIPRIM ANRS Study Group. Intensive five-drug antiretroviral therapy regimen versus standard triple-drug therapy during primary HIV-1 infection (OPTIPRIM-ANRS 147): a randomised, open-label, phase 3 trial. *Lancet Infect Dis*. 2015;15(4):387-396. doi:10.1016/S1473-3099(15)70021-6
12. Markowitz M, Evering TH, Garmon D, et al. A randomized open-label study of 3- versus 5-drug combination antiretroviral therapy in newly HIV-1-infected individuals. *JAIDS J Acquired Immune Defic Syndr*. 2014;66(2):140-147. doi:10.1097/QAI.0000000000000111. PMID: 24457632; PMCID: PMC4123437.
13. Chéret A, Bauer R, Meiffrédy V, et al. Once-daily dolutegravir versus darunavir plus cobicistat in adults at the time of primary HIV-1 infection: the OPTIPRIM2-ANRS 169 randomized, open-label, phase

3 trial. *J Antimicrob Chemother*. 2022;77(9):2506-2515. doi:10.1093/jac/dkac207. PMID: 35762503.

14. Rouzioux C, Hubert JB, Burgard M, et al. Early levels of HIV-1 DNA in peripheral blood mononuclear cells are predictive of disease progression independently of HIV-1 RNA levels and CD4+ T cell counts. *J Infect Dis*. 2005;192:46-55.

15. Tsiara CG, Nikolopoulos GK, Bagos PG, et al. Impact of HIV type 1 DNA levels on spontaneous disease progression: a meta-analysis. *AIDS Res Hum Retroviruses*. 2012;28:366-373.

16. Williams JP, Hurst J, Stöhr W, et al. HIV-1 DNA predicts disease progression and post-treatment virological control. *eLife*. 2014;3:e03821.

17. Geretti AM, Arribas JR, Lathouwers E, et al. Dynamics of cellular HIV-1 DNA levels over 144 weeks of darunavir/ritonavir monotherapy versus triple therapy in the MONET trial. *HIV Clin Trials*. 2013;14:45-50.

18. Alteri C, Scutari R, Stingone C, et al. Quantification of HIV-DNA and residual viremia in patients starting ART by Droplet Digital PCR: their dynamic decay and correlations with immunological parameters and virological success. *J Clin Virol*. 2019;117:61-67.

19. Hocqueloux L, Avettand-Fènoël V, Jacquot S, et al. Long-term antiretroviral therapy initiated during primary HIV-1 infection is key to achieving both low HIV reservoirs and normal T cell counts. *J Antimicrob Chemother*. 2013;68:1169-1178.

20. Besson GJ, Lalama CM, Bosch RJ, et al. HIV-1 DNA decay dynamics in blood during more than a decade of suppressive antiretroviral therapy. *Clin Infect Dis*. 2014;59(9):1312-1321. Epub July 29, 2014. doi:10.1093/cid/ciu585

21. Mondi A, Pinnetti C, Lorenzini P, et al. Virological and immunological outcomes of an intensified four-drug versus a standard three-drug antiretroviral regimen, both integrase strand transfer inhibitor-based, in primary HIV infection. *Pharmaceuticals*. 2022;15(4):403. doi:10.3390/ph15040403

22. Laanani M, Ghosn J, Essat A, et al. Agence Nationale de Recherche sur le Sida PRIMO Cohort Study Group. Impact of the timing of initiation of antiretroviral therapy during primary HIV-1 infection on the decay of cell-associated HIV-DNA. *Clin Infect Dis*. 2015;60(11):1715-1721. doi:10.1093/cid/civ171

23. Hoenigl M, Chaillon A, Moore DJ, et al. Rapid HIV viral load suppression in those initiating antiretroviral therapy at first visit after HIV diagnosis. *Sci Rep*. 2016;6:32947. doi:10.1038/srep32947

24. Nozza S, Poli A, Ripa M, et al. Efficacy of elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate as treatment for primary or recent HIV infection. *J Antimicrob Chemother*. 2017;72(2):632-633. Epub October 17, 2016. doi:10.1093/jac/dkw439

25. Hollingsworth TD, Anderson RM, Fraser C. HIV-1 transmission, by stage of infection. *J Infect Dis*. 2008;198:687-693.

26. Mariaggi AA, Bauer R, Charre C, et al. HIV-1-RNA and total HIV-1-DNA loads in the genital compartment in men receiving dolutegravir- versus darunavir-based combined ART (cART) regimens during primary HIV infection. *J Antimicrob Chemother*. 2022;77(3):735-739. doi:10.1093/jac/dkab427

27. Thornhill J, Inshaw J, Kaleebu P, et al. Brief report: enhanced normalization of CD4/CD8 ratio with earlier antiretroviral therapy at primary HIV infection. *JAIDS J Acquired Immune Defic Syndr*. 2016;73:69-73.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.