Plasma, Intracellular and Lymph node Antiretroviral Concentrations and HIV DNA Change During Primary HIV Infection: Results from the INACTION P25 Study

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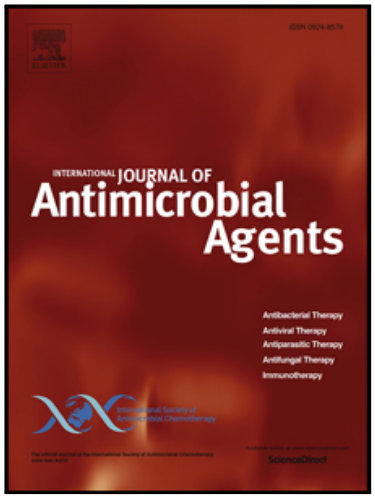

Plasma, Intracellular and Lymph node Antiretroviral Concentrations and HIV DNA Change During Primary HIV Infection: Results from the INACTION P25 Study

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Highlights

- PK/PD study of current regimens during early treatment of primary infection.
- No significant differences between treatment arms have been observed.
- Differences in ARV concentrations in plasma, PBMC and LN were described.
- Higher HIV-DNA decay emerged in patients with low Fiebig stages.
- TFV-DP in PBMC was correlated with HIV-DNA decay in higher Fiebig patients.

Plasma, Intracellular and Lymph node Antiretroviral Concentrations and HIV DNA Change During Primary HIV Infection: Results from the INACTION P25 Study

Amedeo De Nicolò[1*], Alice Palermiti [1*], Stefania Dispinseri[2], Giulia Marchetti[3], Mattia Trunfio[4], Elisa De Vivo[1], Antonio D'Avolio[1], Antonio Muscatello [5], Andrea Gori[5], Stefano Rusconi[6,7], Elena Bruzzesi[8], Arianna Gabrieli[9], Davide Paolo Bernasconi[10], Alessandra Bandera[7], Silvia Nozza[11], Andrea Calcagno[4] *on behalf of the INACTION (Italian Network of Acute HIV Infection) study group*.

*These authors contributed equally to this paper

1. Laboratory of Clinical Pharmacology and Pharmacogenetics, Department of Medical Sciences, University of Turin, Turin;
2. Viral Evolution and Transmission Unit, IRCCS Ospedale San Raffaele, Milan;
3. Clinic of Infectious Diseases, Department of Health Sciences, ASST Santi Paolo e Carlo, University of Milan, Milan;
4. Unit of Infectious Diseases, Department of Medical Sciences, University of Turin, Turin;
5. Infectious Diseases Unit, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan;
6. UOC Malattie Infettive, Ospedale Civile di Legnano, ASST Ovest Milanese, Legnano;
7. University of Milan, Milan;
8. Vita-Saluta San Raffaele University, Milan;
9. Dipartimento di Scienze Biomediche e Cliniche, Ospedale L Sacco, Milan;
10. Bicocca Bioinformatics Biostatistics and Bioimaging Centre - B4 School of Medicine and Surgery, University of Milano-Bicocca, Monza;
11. Department of Infectious Diseases, IRCCS Ospedale san Raffaele, Milan, Italy.

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Corresponding Author: Dr. Amedeo De Nicolò; e-mail: amedeo.denicolo@unito.it; Phone: +390114396867
Laboratory of Clinical Pharmacology and Pharmacogenetics*.
University of Turin, Department of Medical Sciences, Amedeo di Savoia Hospital, Corso Svizzera 164 - 10149 - TURIN (ITALY)
*UNI EN ISO 9001 and 13485 Certified Laboratory;
Web Site: www.tdm-torino.org

Abstract

Despite its high effectiveness, combination antiretroviral treatment (cART) has a limited effect on HIV-DNA reservoir, which establishes early during primary infection (PHI) and is maintained by latency, homeostatic T-cells proliferation, and residual replication: this can be associated with low drug exposure in lymphoid tissues and/or suboptimal adherence to antiretroviral drugs (ARVs). Aim of this study was to assess ARVs concentrations in plasma, peripheral blood mononuclear cells (PBMC) and lymph nodes (LN), and their association to HIV-RNA and DNA decay during PHI. Participants were randomized to receive standard doses of darunavir/cobicistat (arm I), dolutegravir (arm II) or both (arm III), with a backbone of tenofovir alafenamide and emtricitabine. Total HIV-DNA was measured by digital-droplet PCR in PBMC at baseline, 12 and 48 weeks. Plasma and PBMC drugs concentrations were determined at 2, 12 and 48 weeks (LN at 12 weeks) by UHPLC-MS/MS. Seventy-two participants were enrolled, mostly male (n=68), median age 34 years and variable Fiebig stages (V-VI 57.7%, I-II 23.9%, and III-IV 18.3%). Twenty-six patients were assigned to Arm I, 27 to Arm II and 19 to Arm III. After 48 weeks, most patients had undetectable viremia, with minor between-arms differences in HIV-RNA decay. Patients with Fiebig I-II showed faster HIV-RNA and HIV-DNA decay. Intracellular-tissue penetration was high for nucleoside analogues and low-moderate for darunavir and dolutegravir. Only tenofovir diphosphate concentrations in PBMC showed correlation with HIV-DNA decay. Overall, this study suggests the timing of treatment initiation and intracellular tenofovir penetration as primary and secondary factors affecting HIV reservoir.

Keywords: Acute HIV infection, Primary HIV infection, Antiretroviral therapy, Intracellular, Tissue, Pharmacokinetics.

1. Introduction

The persistence of HIV in cells and in protected organs and tissues is one of the key reasons why HIV can be pharmacologically controlled but not eradicated in people living with HIV (PLWH) [1-4]. There are different factors which sustain HIV persistence during cART, including proviral HIV-DNA latency, the homeostatic proliferation of infected T-cells and residual replication [3, 5-9], leading to an extremely slow and limited decay of the HIV DNA reservoir during treatment [1, 10, 11] Some curative strategies are currently tested, in some cases with contrasting approaches: these include the reactivation of latently infected cells along with immune system enhancing treatments, known as the "kick and kill" strategy [12, 13], or the epigenetic inhibition of viral transcription, known as "block and lock" approach [14-16], as well as "gene editing" therapies, aiming at modifying proviral DNA to make it replication incompetent [8, 17-20]. Despite significant research efforts, up to date only allogenic bone marrow transplant from donors with homozygous CCR5 gene deletion (in 3 individuals to date) [21-24], and treatment during acute infection have been associated with virological control after treatment interruption [25-28]. Primary HIV infection (PHI) is a rarely diagnosed condition where early treatment has been associated with improved clinical, immunological, and virological outcomes and, in approximately 8% (5 to 15%) of patients in a French cohort, post-treatment control (i.e. virological control without ARVs after a period of cART treatment) [27, 28]. Albeit promising, these results only highlighted a subset of patients that received antiretroviral treatment (ART) during PHI: randomized and controlled studies did not identify the most beneficial therapeutic regimen for patients diagnosed early after being infected by HIV [29].

Several tissues and organs have been listed as potential sites of continuous, and potentially differential, replication during suppressing ART (including central nervous system, lymph

nodes, spleen, and gut/gut-associated lymphoid tissue) [2, 4, 6, 8, 30-32]. Recent studies have identified lymphoid tissues as sanctuary sites where antiretroviral penetration is limited and variable and where HIV replication may persist [2, 8, 31, 33]. Physicochemical characteristics associated with greater lymphatic system penetration were shown to be high molecular weight, larger particle size, log P value >5, high long chain triglycerides solubility [19, 31]. Additional features potentially affecting drug passage in LNs are tissue fibrosis (observed in several PLWH), inflammation and transporter expression and activity [34-36]. Currently published studies, based on different methods (hollow fibers, models, tissue homogenate, mononuclear cell extraction), suggested differential exposures in lymph nodes as well as highly variable inhibitory quotients [30, 31, 37, 38]. A trend towards lower LN HIV RNA was observed in the follow up of patients treated during PHI with 2 nucleos(t)ide reverse transcriptase inhibitors (NRTIs) plus dolutegravir (DTG) and maraviroc: the latter CCR5-inhibitor showed a peculiarly high LN penetration [30]. Aims of this sub-study were to quantify ARV concentrations in plasma, peripheral blood mononuclear cells (PBMC) and lymph nodes (LN) and to evaluate their association with HIV DNA decay up to week 48 after ART introduction in people treated during PHI with currently used potent (and common) combinations.

2. Material and methods

2.1. Enrolment, inclusion criteria and randomization

Treatment naive adult participants diagnosed with PHI and who gave written informed consent were included in this prospective, randomized, open-label, multicentre clinical study, which received ethical approval from the Institutional Ethics Committee of the San Raffaele Hospital (Coordinating centre).

Inclusion and exclusion criteria were described in detail in a previous work[39]: in particular, the self-reported adherence to the treatment had to be higher than 95% throughout the study period. Moreover, an additional exclusion criterion was the observation of detectable tenofovir alafenamide (TAF) concentrations in plasma throughout the protocol, which should be not detectable at the end of dosing interval (24 hours, C_{trough}) since it is known to be rapidly converted during the first 8 hours after the dose intake [40]. Its detection in plasma can be a marker of suboptimal adherence to the timings of drug intake in the study protocol.

Patients were randomized in a proportion 10:10:8 for receiving one among the following three different treatment regimens, all sharing a TAF and emtricitabine (FTC) nucleosidic backbone: darunavir boosted with cobicistat (DRV + COBI 800/150 mg QD; DRV/c) plus TAF/FTC 10/200 mg QD; dolutegravir (DTG, 50 mg QD) plus TAF/FTC 25/200 mg QD; DTG + DRV/c + TAF/FTC (800/150 mg QD + 50 mg QD + 10/200 mg QD, respectively). The reason for investigating these combinations was that they are: in case of DTG, first-line, with good distribution, high barrier to resistance and expected fast virological decline; in case of DRV, good CSF penetration, high barrier to resistance and post-transcriptional effect.

2.2. Clinical and Virological Follow-up

Participants underwent a strict monitoring of the virological and immunological parameters throughout the protocol. Plasma HIV-RNA viral load (Limit of quantification, LLOQ 25 copies/mL, Limit of detection, LOD 20 copies/mL), as well as CD4+ lymphocytes count, were measured at baseline and then at 2, 4, 8, 12, 24, 36, 48 weeks of therapy. HIV/RNA viral loads lower than the LLOQ were approximated to the LOD value of 20 copies/mL. HIV total proviral DNA (hereafter, HIV DNA) was quantified in PBMC purified by density gradient, by a

validated digital droplet PCR method (LLOQ 2 copies/million cells), at the baseline, 12 and 48 weeks.

2.3. Drugs quantification in plasma, PBMC and Lymph nodes

All enrolled patients underwent blood sampling for the quantification of drug concentrations in plasma at the end of the dosing interval (C_{trough}) at 2, 4, 8, 12, 24, 36 and 48 weeks. Intra-PBMC concentrations were determined, conversely, at 2, 12 and 48 weeks of treatment. PBMC isolates were obtained by density gradient with CPT tubes, capable to strongly reduce the contamination by red blood cells, with a protocol with 2 fast washing steps with 0.9% NaCl at 4°C, as previously described [41, 42].

The analytes were measured by validated UHPLC-MS/MS methods [42-44], based on the protein precipitation and the use of stable isotope labelled internal standards (SIL-IS) for each analyte. LLOQ values for each drug in plasma and PBMC were, respectively: 31 ng/mL and 0.39 ng/sample for DTG, 39 ng/mL and 0.039 ng/sample for DRV, 10 ng/mL and 0.039 ng/sample for COBI. On the other hand, the quantification of TFV-diphosphate (TFV-DP) and FTC-triphosphate (FTC-TP) in PBMC and lymph nodes was performed by a validated HPLC-MS/MS method based on ion coupling chromatography on a graphitic carbon column (Hypercarb ® 3 µm, 2.1 x 100 mm column) with a gradient of 2 mobile phases: water + 0.2% hexylamine and 5 mM of Diethylamine + 2% of Acetic Acid (Mobile Phase A); and Acetonitrile/mobile phase A in a proportion of 60:40 vol:vol (Mobile Phase B). The gradient run duration was 15 minutes at 40°C and flow rate of 0.4 mL/min.

The method was validated following FDA and EMA guidelines on PBMC samples, showing satisfactory accuracy (trueness, bias <15%) and intra- and inter-day precision (coefficients of

variation <15%), and reproducible matrix effect and recovery. The LLOQ for TFV-DP and FTC-TP was 0.039 ng/sample.

Intracellular drug amounts were then normalized by volume of cells in each sample, calculated by multiplying the cell numbers and a mean cell volume of 283 fL, as described previously [41], obtaining intracellular concentrations, in ng/mL. Correcting the LLOQ values for the intracellular assays by a mean cell number per sample of 10 million (volume of 2.8 µL/sample), the intracellular LLOQ for all the drugs was 13.8 ng/mL.

The quantification in needle biopsies (approximately about 10 mg of tissue) from inguinal lymph nodes was performed only in a small subset of patients who agreed to undergo needle biopsies for viro-immunological and pharmacological purposes at 12 weeks of treatment, at the end of dosing interval. In these samples, the quantification was performed after homogenization, protein precipitation and UHPLC-MS/MS analysis. The observed drug amounts have been corrected by sample weights, to obtain concentrations in ng/g.

To compare LN to plasma and LN to PBMC ratios, an approximate density for LN of 1 g/mL was assumed, in accordance with a previous work and confirmed experimentally in our samples [45]. For the determination of intracellular TFV-DP and FTC-TP to plasma TFV and FTC concentrations ratios, all the results were corrected by the molar ratios (TFV-DP/TFV and FTC-TP/FTC) of 0.642 and 0.507, respectively.

2.4. Statistical Analysis

The distribution of continuous variables was summarized using median and first-third quartile (IQR) while categorical variables were reported as absolute number and percentage. Patients' characteristics and virological parameters were compared between

treatment arms using Kruskal-Wallis or Chi-square tests for continuous and categorical variables, respectively.

Friedman test was adopted to test the difference of the drug concentrations between time visits (weeks 2, 12 and 48), while Spearman correlation index was used to evaluate the association between drug concentrations and HIV DNA change in time (at weeks 12 and 48).

Finally, the association between Fiebig stage (I-II vs III-IV vs V-VI) and the change in time of log-transformed HIV RNA (baseline, week 4, 8, 12, 24, 36, 48) and log-transformed HIV-DNA (baseline, week 12 and 48) was assessed using mixed effects linear regression with participant as random effect.

3. Results

3.1 Patients' baseline characteristics

A total number of 78 patients were screened and 72 patients met inclusion criteria. The 6 excluded patients showed detectable TAF concentrations in plasma, thus they were excluded from the analysis due to suspected suboptimal adherence to the timing of drug intake according to the protocol. Patients' characteristics at baseline are reported in table 1.

Most patients were male (68, 94.4%) and relatively young, with a median age of 34.1 years (IQR 28.3 – 43.9). Median baseline CD4+ cell count was 658 cells/mL (IQR 474 – 796); median HIV-RNA in plasma was 5.66 Log_{10} cps/mL (IQR 4.62 – 6.50) while HIV-DNA in PBMCs was 4.46 Log_{10} cps/10^6 PBMC (IQR 4.08 – 4.81).

Patient were mostly diagnosed during Fiebig stages V-VI (57.7%), I-II (23.9%) and, finally, III-IV (18.3%).

3.2 Between Arms differences

After randomization, 26 patients were assigned to the DRV/c arm (Arm I), 27 to the DTG arm (Arm II) and 19 to the DTG + DRV/c arm (Arm III). There were no significant differences in baseline characteristics between study arms, as well as in the CD4+ T-cell count reconstitution up to 48 weeks or in HIV DNA change over time. Arm I (DRV) showed a mildly slower decay in plasma HIV RNA during the first 12 weeks compared with the others. No significant differences were observed in drugs concentrations (for TFV and FTC), neither in plasma, PBMC or LN between study arms. Detailed data about differences between regimens have been published elsewhere [39].

3.3 Drug concentrations in the compartments and correlation with virological effectiveness

Median trough concentrations in plasma, PBMCs and LNs (in a small subset of patients, n=9) for each ARV are summarized in Table 2 (PBMC- and tissue-to-plasma ratios are described in supplementary material). DTG plasma concentrations tended to increase over time, showing slightly higher concentrations at weeks 12 and 48 ($p = 0.097$), but this trend was not present in PBMC ($p = 0.646$). All samples showed quantifiable drug concentrations (except for TAF).

DTG and DRV penetration in PBMC appeared limited, showing median ratios of 0.31 and 0.32, respectively, at 12 weeks of treatment. Conversely, TFV-DP and FTC-TP showed higher penetration in PBMC (median ratios 28.29 and 16.98, respectively) and whole-LN (median ratios of 11.13 and 16.04, respectively).

Drug concentrations in plasma and PBMC resulted mutually correlated for DTG, DRV and COBI (Spearman rho values between 0.366 and 0.789, P values between 0.073 and < 0.001). Conversely, concentrations of TFV-DP and FTC-TP in PBMCs were not correlated to those in plasma (P values > 0.075) at any time point.

3.4 PK/PD correlations

Testing the correlation between drug concentrations in plasma, PBMC and LN with HIV RNA and HIV-DNA viral load change over time showed only borderline correlations of HIV DNA change at 48 weeks with TFV-DP concentration in PBMC ($r = -0.32$; $p = 0.057$) and TFV-DP accumulation ratio in PBMC/plasma ($r = -0.33$; $p = 0.053$), suggesting that higher intracellular accumulation of TFV-DP was associated with higher decay in HIV DNA.

3.5 Effect of Fiebig stages on immunological/virological endpoints

As reported in Table 1, participants were widely variable in terms of Fiebig stage: the most frequent stages were II (13 patients, 18.3%), V (29 patients, 40.8%) and VI (12 patients, 16.9%). No significant differences were observed between Fiebig stages in terms of baseline CD4+ cells count and recovery over time ($p > 0.641$). On the other hand, participants with lower stages (I-II) had significantly higher plasma HIV RNA at baseline ($p = 0.001$) compared with participants diagnosed at later stages (III-VI); this difference was lost at follow up, resulting in a significantly higher decay of plasma HIV RNA in participants diagnosed at Fiebig stages I-II (Figure 1, $p = 0.031$ vs III-IV and $p < 0.001$ vs V-VI).

Conversely, no differences were observed in terms of HIV DNA in PBMC samples between Fiebig stages at baseline ($p = 0.739$), but this difference became more and more important during treatment, reaching a borderline significance at 48 weeks of treatment ($p = 0.068$). This significant trend was confirmed by the mixed modelling, that showed a significantly faster HIV DNA decay during the treatment in patients with Fiebig stages I-II compared to stages III-IV ($p = 0.017$) and V-VI ($p = 0.005$), respectively. These results are depicted in Figure 2.

3.6 Interplay between Fiebig stages and cART

Once the single impacts of treatment arms and Fiebig stages on the virological endpoints were observed, we aimed to understand the effect of the cART, in terms of type of treatment and PK features, stratifying the participants in low (I-II) and high (III-VI) Fiebig stages. In table 3 and 4 patients characteristics during treatment are summarized, divided by treatment arm in low (Table 3) and high (Table 4) Fiebig stages.

As can be seen, no significant differences in terms of CD4+ cell recovery, HIV RNA and HIV DNA kinetics were found between treatment arms in low Fiebig stages, while a significantly slower decay in HIV RNA viral load was observed in patients from Arm I (DRV) as compared with other arms (DTG and DRV+DTG) in the patients with higher Fiebig stages (III-VI). In line with the previous associations between HIV DNA decay and PK/PD parameters, only TFV-DP concentration in PBMC ($r = -0.46$; $p = 0.019$) and the TFV-DP PBMC/plasma concentration ratio ($r = -0.47$; $p = 0.017$) were correlated with HIV DNA decay at week 48 in the higher Fiebig stages group. This correlation was depicted in Figure 3.

4. Discussion

The present randomized and controlled study is one the first evaluating modern therapeutic choices for the treatment of PHI and to confirm the lack of effect of intensified regimens. The results from our study confirm the evidence from previous cohorts regarding the higher HIV DNA decay in PHI, particularly during the very early phases, namely Fiebig stages I-II [25].

Comparing the decay in the HIV RNA and DNA among the study arms, we observed a significantly faster decay in HIV RNA in the arms containing DTG (arm II and III), but no

difference concerning HIV DNA decay. This evidence was more evident in the context of more advanced Fiebig stages (III-VI), while not statistically significant in extremely early stages (Fiebig I-II).

From the PK point of view, we observed that the drugs which showed the highest penetration in PBMC and in LN were the phosphorylated metabolites of the nucleoside analogues, TFV-DP, and FTC-TP, confirming previous reports and the well-known capability of TAF to accumulate in PBMCs [19, 37, 46]. On the other hand, both the "third drugs" (either DTG or DRV) showed PBMC/plasma and LN/plasma ratios lower than 1, highlighting a moderate penetration of these drugs in PBMC and in LN: these results are evidently higher (0.32 and 0.59, respectively) than those reported by Fletcher *et al.* in LN mononuclear cells (LNMC) [19], where the reported DTG LNMC/plasma ratio was nearly 0.01. This difference was expected, since these studies aimed at describing drug concentrations in two different compartments: in our work, we used tissue homogenates from inguinal LN to describe the drug concentrations in the whole LN microenvironment, including interstitial fluid and different cell types, so the results cannot be directly comparable to the intra-LNMC concentrations, particularly for drugs with limited intracellular penetration (such as DTG). This important difference between concentrations in LN homogenates (generally lower than concentrations in plasma for the "third drugs"), representing the microenvironment, and the literature-reported LNMC concentrations confirm lower than proportional penetration of DTG from the extracellular matrix in LNMC compared to PBMC (from blood/plasma): on the other hand, it is important mentioning that the quantification of DTG and other drugs in LNMC needs some steps of cell isolation with aqueous buffers (eg. use of cell strainers with cell washings) [31] which are potentially associated with drug loss from the cells, possibly leading to a slight underestimation of DTG concentrations.

Interestingly, the observed LN/plasma concentrations ratios for DTG were comparable (0.59 vs 0.44) with other studies which considered lymphoid tissues homogenates, such as the one by Weber *et al.* on gut-associated lymphoid tissue (GALT) [47]. On the opposite, concerning TFV-DP and FTC-TP, there is less significant impact of the extracellular matrix on the intracellular quantification, since these metabolites are only produced and tend to remain within the cells, due to their highly hydrophilic nature. Interestingly, by a PK/PD point of view, only TFV-DP concentrations in PBMC showed a significant association with HIV DNA decay after 48 weeks of treatment, in accordance with previous reports, while others did not [31, 36, 48]. TAF is a prodrug specifically designed to show high PBMC/plasma accumulation ratios; moreover TFV-DP, the active intracellular moiety, has a well-known long half-life (range 30 – 72 hours), which makes it a marker of the overall exposure to the treatment [40, 49, 50]. Therefore, combining these characteristics, the correlation between HIV DNA decay and TFV-DP accumulation within PBMC may be explained by a higher cumulative exposure to TAF, including higher overall bioavailability (absorption and first-pass metabolism) and/or higher adherence during the study period [49]. Moreover, it is worth to note how the impact of TFV-DP concentrations in PBMC resulted significant only in the group of participants with more advanced Fiebig stages (III-VI), probably suggesting that a higher HIV DNA decay can be achieved with extremely early treatment, relatively irrespective of drug concentrations, while higher PK exposure may be required to achieve a similar goal in less recent infections.

It is interesting to note a gradual trend for DTG concentrations in plasma and PBMC to increase over time throughout the protocol: considering that all the measurements were performed at the steady state, this trend may be explained by a gradual change in the inflammatory status in these patients, in accordance with previous reports [35, 51].

Nevertheless, the present study has some limitations: the PK evaluation has been performed only in a small subset of patients (n=9) in LN, due to the invasive sampling, and the quantification in LN was performed in tissue homogenates, including different cell types and interstitial fluid, being therefore not comparable with previously described drug concentrations in LNMC; total HIV DNA was quantified, which is expected to be the most reliable by a quantitative point of view [7, 10, 52], but we cannot infer about its replication competency; finally, the overall relatively low number of patient and the inhomogeneous treatments cannot allow us to apply enough stratification to draw definitive conclusions.

Concluding, the present study suggests variable ARVs penetration in PBMC and LN compartments, confirming higher penetration and impact on HIV DNA reservoir for TFV-DP, particularly compared with DTG and DRV. Anyway, it is worth noting that this correlation emerges lately during the follow-up (at 48 weeks) and is particularly marked in patients with less recent PHI (Fiebig III-VI), suggesting a cumulative and slow effect of TFV-DP on HIV-DNA during the first year of treatment. Moreover, it cannot be excluded that the observed correlation between HIV-DNA change and TFV-DP can be explained by a mutual correlation with a third genetic or immunological factor we did not observe. This latter point deserves further investigation. On the other hand, extremely early treatment, during Fiebig stages I-II, overcomes the impact of treatment regimens and ARV PK in PBMC on HIV DNA decay, highlighting the timing of treatment initiation and the treatment itself (regimen and exposure) as primary and secondary factors, respectively, to affect HIV reservoir.

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Declarations

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Competing Interests: Amedeo De Nicolò, Alice Palermiti, Stefania Dispinseri, Elena Bruzzesi, Arianna Gabrieli, Mattia Trunfio, Elisa De Vivo, Antonio D'Avolio and Davide Paolo Bernasconi have no conflict of interest to declare. Silvia Nozza, Stefano Rusconi, Giulia Marchetti, Andrea Calcagno, Antonio Muscatello, Alessandra Bandera, received fees for Advisory Boards and consultations from ViiV Healthcare.

Ethical Approval: The study was approved by the Ethical Committee, Ospedale San Raffaele, Milan, Italy. All participants signed informed consent.

Sequence Information: No

Randomized Controlled Trial: This study is registered under the number NCT04225325 on ClinicalTrials.gov.

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.

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Figure legends

Figure 1: HIV RNA changes throughout the treatment period of 48 weeks, stratified by Fiebig stages.

Figure 2: HIV DNA changes in peripheral blood mononuclear cells (PBMC) during the study period, stratified by Fiebig stages.

Figure 3: Correlation between the HIV DNA change during the study period and the intra-PBMC TFV-DP concentration in the group of patients with higher Fiebig stages (Fiebig III to VI).

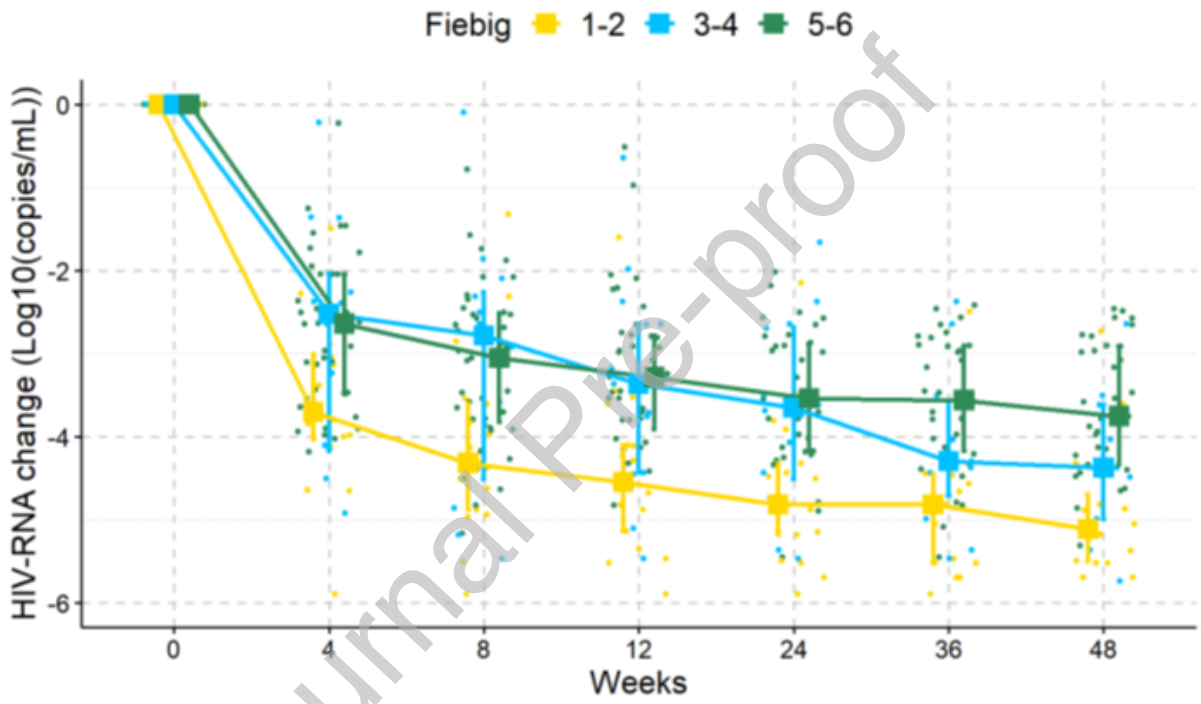

Fig 1

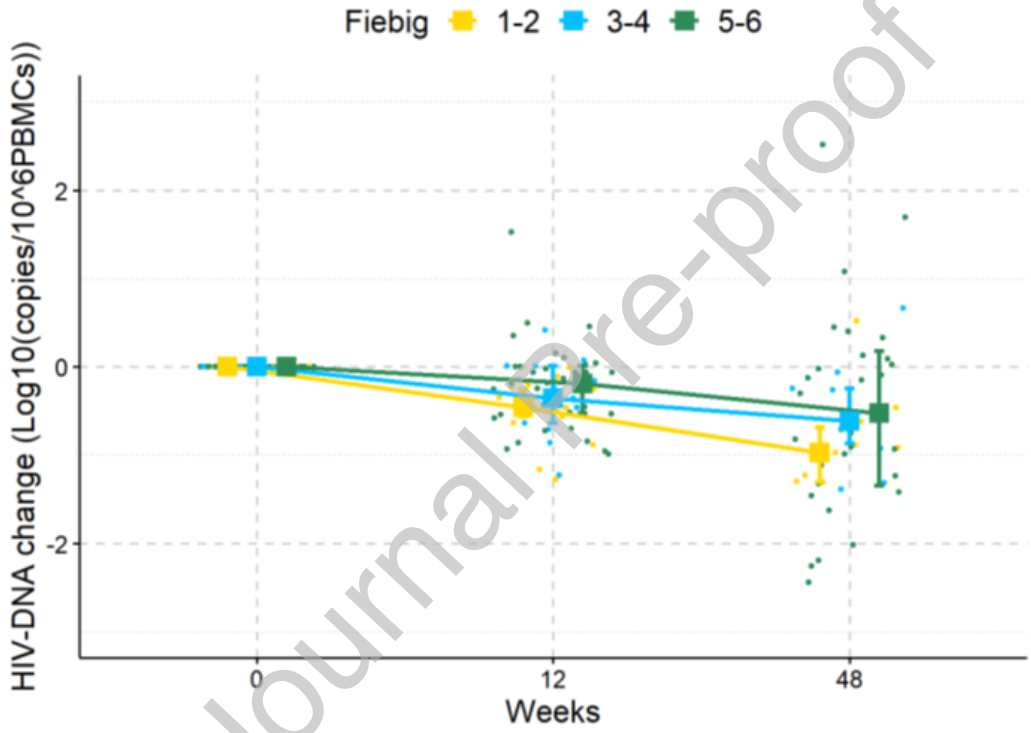

Fig 2

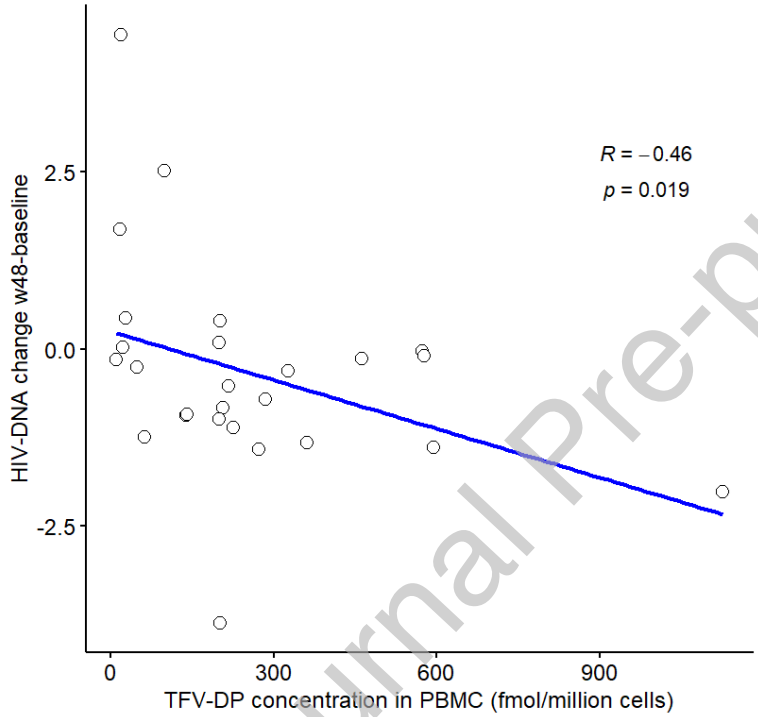

Fig 3

Table 1: Participants characteristics at the baseline.

Total n. of participants (n = 72)	
Randomization Arms Arm I: DRV/c + TAF + FTC Arm II: DTG + TAF + FTC Arm III: DTG + DRV/c + TAF + FTC	 I = 26 II = 27 III = 19
Sex (male/female)	68/4 (94.4% / 5.6%)
Age (median, IQR)	34.1 (28.3 – 43.9)
Sexuality (n; %)	Heterosexual/Bisexual = 21 (29.2%) MSM = 42 (58.3%) Not reported = 9 (12.5%)
Fiebig Stage (n; %)	I = 4 pt (5.5%) II = 13 pt (18.1%) III = 5 (6.9%) IV = 9 (12.5%) V = 29 (40.3%) VI = 12 (16.7%)
CD4+ count (cells/mm^3)	658 (IQR 474 – 796)
Plasma HIV-RNA (Log_{10} cps/mL)	5.66 (IQR 4.62 – 6.50)
Plasma HIV-DNA (Log_{10} cps/mL)	4.46 (IQR 4.08 – 4.81)

Table 2: Summary of the observed drugs' concentrations in plasma, PBMC and in LN homogenates throughout the protocol.

Drug	Week 2			Week 12				Week 48		
	Plasma (ng/mL)	PBMC (ng/mL)	PBMC (fmol/10^6 cells)	Plasma (ng/mL)	PBMC (ng/mL)	PBMC (fmol/10^6 cells)	LN* (ng/g)	Plasma (ng/mL)	PBMC (ng/mL)	PBMC (fmol/10^6 cells)
Median DTG conc. (IQR)	1213 (465 – 1911)	478 (114 -1422)	323 (77 – 960)	1613 (1285 – 2306)	592 (301 – 1402)	400 (203 – 946)	851 (482 – 2530)	1817 (1278 – 2344)	630 (381 – 1090)	425 (257 – 736)
Median DRV conc. (IQR)	2144 (1053 – 3191)	984 (403 – 4125)	509 (208 – 2132)	1887 (821 – 3192)	783 (285 – 1327)	405 (147 – 686)	983 (253 – 1890)	2120 (709 – 3700)	983 (281 – 2216)	508 (145 – 1146)
Median COBI conc. (IQR)	97 (35 – 382)	379 (111 – 1797)	138 (41 – 656)	126 (43 – 267)	299 (85 – 513)	109 (31 – 187)	49 (11 – 113)	188 (32 – 316)	798 (90 – 1199)	291 (32 – 438)
Median TFV conc. (IQR)	8.5 (7.2 – 11.5)	500 (202 – 778)	318 (128 – 494)	7.9 (6.5 – 10.6)	379 (190 – 576)	241 (121 – 366)	170 (49 – 452)	9.4 (7.8 – 12.8)	316 (100 – 556)	201 (64 – 353)
Median FTC conc. (IQR)	126 (56 – 195)	4074 (2432 – 6822)	2367 (1412 – 3964)	109 (68 – 202)	4330 (2120 – 6152)	2516 (1232 – 3574)	2532 (253 – 3667)	125 (75 – 285)	4342 (2282 – 7443)	2522 (1326 – 4324)

* LN concentration was determined on the whole-tissue homogenate, including intracellular and interstitial fluid concentrations. No normalization by cell numbers can be performed.

Table 3: Descriptive statistics stratified by randomization arm, in patients with early (I-II) Fiebig stages. P values are referred to Kruskal-Wallis or Chi-square tests for continuous and categorical variables, respectively.

Factors	Level/Unit	Fiebig I-II			P value
		TAF/FTC + DRV N=6	TAF/FTC + DTG N=6	TAF/FTC + DRV + DTG N=5	
Gender (%)	F	1 (16.7)	0 (0.0)	0 (0.0)	0.378
	M	5 (83.3)	6 (100.0)	5 (100.0)	

Age (median [IQR])	y.o.	32.1 [28.8 - 40.4]	39.3 [33.7 - 48.2]	44.8 [36.7 - 47.3]	0.714
HBsAg (%)	no	6 (100.0)	6 (100.0)	5 (100.0)	NA
HCV (%)	no	6 (100.0)	6 (100.0)	5 (100.0)	NA
median CD4 BL [IQR]	Cells/mm^3	779 [633 - 794]	570 [500 - 658]	567 [472 - 677]	0.37
median CD4 w12 [IQR]	Cells/mm^3	745 [606 - 885]	546 [496 - 599]	642 [515 - 689]	0.134
median CD4 w24 [IQR]	Cells/mm^3	721 [616 - 842]	630 [570 - 640]	659 [523 - 707]	0.61
median CD4 w24 [IQR]	Cells/mm^3	957 [813 - 1065]	730 [712 - 809]	665 [538 - 714]	0.088
median CD4 w48 [IQR]	Cells/mm^3	880 [768 - 1016]	685 [609 - 712]	596 [529 - 643]	0.056
median HIV RNA BL [IQR]	Log_{10} cps/mL	6.89 [6.28 - 7.00]	6.61 [6.50 - 6.91]	7.00 [6.11 - 7.00]	0.916
median HIV RNA w2 [IQR]	Log_{10} cps/mL	3.89 [3.39 - 5.15]	3.82 [3.22 - 4.41]	3.24 [2.65 - 3.55]	0.405
median HIV RNA w4 [IQR]	Log_{10} cps/mL	3.37 [3.06 - 4.11]	2.85 [2.35 - 3.51]	2.36 [2.35 - 2.73]	0.194
median HIV RNA w8 [IQR]	Log_{10} cps/mL	3.03 [1.80 - 4.03]	2.52 [1.81 - 2.66]	2.04 [1.49 - 2.06]	0.299
median HIV RNA w12 [IQR]	Log_{10} cps/mL	2.26 [1.44 - 3.22]	2.19 [1.80 - 2.31]	1.53 [1.48 - 1.65]	0.184
median HIV RNA w24 [IQR]	Log_{10} cps/mL	1.74 [1.38 - 2.61]	2.10 [1.72 - 2.15]	1.48 [1.41 - 1.51]	0.52
median HIV RNA w36 [IQR]	Log_{10} cps/mL	1.39 [1.30 - 2.25]	1.79 [1.60 - 2.09]	1.30 [1.30 - 1.48]	0.199
median HIV RNA w48 [IQR]	Log_{10} cps/mL	1.48 [1.30 - 1.60]	1.61 [1.60 - 1.67]	1.48 [1.30 - 1.51]	0.4
median HIV DNA BL [IQR]	Log_{10} cps/10^6 PBMCs	4.18 [3.91 - 4.82]	4.42 [4.17 - 4.73]	4.49 [4.45 - 4.77]	0.777
median HIV DNA w12 [IQR]	Log_{10} cps/10^6 PBMCs	3.68 [3.50 - 4.10]	3.96 [3.41 - 4.18]	4.24 [3.85 - 4.34]	0.612
median HIV DNA w48 [IQR]	Log_{10} cps/10^6 PBMCs	3.49 [0.83 - 3.74]	3.82 [3.24 - 4.18]	3.57 [1.78 - 3.84]	0.486

Table 4: Descriptive statistics stratified by randomization arm, in patients with later (III-VI) Fiebig stages. P values are referred to Kruskal-Wallis or Chi-square tests for continuous and categorical variables, respectively.

Factors	**Level/Unit**	**Fiebig stages III-VI**			**P value**
		TAF/FTC + DRV N=20	**TAF/FTC + DTG N=21**	**TAF/FTC + DRV + DTG N=13**	
Gender (%)	F	2 (10.0)	0 (0.0)	0 (0.0)	0.171

	M	18 (90.0)	21 (100.0)	13 (100.0)	
Median age [IQR]		34.64 [29.62, 44.13]	31.56 [27.75, 37.76]	32.48 [27.25, 46.28]	0.769
HBsAg. (%)	no	19 (100.0)	20 (100.0)	11 (100.0)	NA
HCV (%)	no	17 (94.4)	18 (94.7)	12 (100.0)	0.712
	yes	1 (5.6)	1 (5.3)	0 (0.0)	
median CD4 BL [IQR]	Cells/mm^3	524 [444, 722]	706 [517, 915]	687 [482, 781]	0.296
median CD4 w12 [IQR]	Cells/mm^3	613 [449, 815]	720 [472, 920]	641 [590, 885]	0.784
median CD4 w24 [IQR]	Cells/mm^3	580 [508, 668]	706.00 [531, 805]	742 [545, 940]	0.21
median CD4 w24 [IQR]	Cells/mm^3	676 [557, 877]	836 [618, 1003]	842 [611, 968]	0.357
median CD4 w48 [IQR]	Cells/mm^3	695 [567, 887]	683 [561, 980]	715 [519, 1004]	0.988
median HIV RNA BL [IQR]	Log_{10} cps/mL	4.86 [4.17, 5.75]	5.28 [4.26, 5.74]	5.95 [4.88, 6.29]	0.196
median HIV RNA w2 [IQR]	Log_{10} cps/mL	3.45 [2.68, 3.92]	2.16 [1.82, 2.64]	2.79 [2.46, 3.07]	0.002
median HIV RNA w4 [IQR]	Log_{10} cps/mL	3.22 [2.54, 3.57]	1.65 [1.39, 2.19]	2.13 [1.98, 2.18]	<0.001
median HIV RNA w8 [IQR]	Log_{10} cps/mL	2.74 [2.09, 3.05]	1.49 [1.30, 1.60]	1.60 [1.55, 1.81]	<0.001
median HIV RNA w12 [IQR]	Log_{10} cps/mL	2.20 [1.61, 2.47]	1.30 [1.30, 1.57]	1.60 [1.57, 2.15]	0.001
median HIV RNA w24 [IQR]	Log_{10} cps/mL	1.60 [1.30, 1.89]	1.30 [1.30, 1.55]	1.56 [1.30, 1.62]	0.132
median HIV RNA w36 [IQR]	Log_{10} cps/mL	1.30 [1.30, 1.39]	1.30 [1.30, 1.40]	1.57 [1.30, 1.63]	0.255
median HIV RNA w48 [IQR]	Log_{10} cps/mL	1.30 [1.30, 1.35]	1.30 [1.30, 1.39]	1.30 [1.30, 1.61]	0.761
median HIV DNA BL [IQR]	Log_{10} cps/10^6 PBMCs	4.39 [4.01, 4.66]	4.57 [4.14, 4.88]	4.40 [4.08, 4.93]	0.702
median HIV DNA w12 [IQR]	Log_{10} cps/10^6 PBMCs	4.12 [3.83, 4.31]	4.34 [3.96, 4.63]	4.29 [3.80, 4.56]	0.228
median HIV DNA w48 [IQR]	Log_{10} cps/10^6 PBMCs	3.90 [3.62, 4.53]	3.95 [3.52, 4.44]	4.16 [3.78, 4.38]	0.893

Graphical Abstract