

## Pre-ART HIV-1 DNA in CD4+ T cells correlates with baseline viro-immunological status and outcome in patients under first-line ART

Francesca Ceccherini-Silberstein<sup>1\*</sup>, Alessandro Cozzi Lepri<sup>2</sup>, Claudia Alteri<sup>1</sup>, Esther Merlini<sup>3</sup>, Matteo Surdo <sup>1,†</sup>, Giulia Marchetti<sup>3</sup>, Maria Rosaria Capobianchi<sup>4</sup>, Andrea De Luca<sup>5</sup>, Nicola Gianotti<sup>6</sup>, Pierluigi Viale<sup>7</sup>, Massimo Andreoni<sup>1</sup>, Andrea Antinori<sup>5</sup>, Carlo Federico Perno<sup>4,8</sup> and Antonella d'Arminio Monforte<sup>3</sup> on behalf of the ICONA Foundation Study Group‡

<sup>1</sup>Department of Experimental Medicine and Surgery, University of Rome Tor Vergata, Rome, Italy; <sup>2</sup>Centre for Clinical Research, Epidemiology, Modelling and Evaluation (CREME) Institute for Global Health, UCL, London, UK; <sup>3</sup>Department of Health Sciences, Institute of Infectious and Tropical Diseases, ASST Santi Paolo e Carlo, University of Milan, Milan, Italy; <sup>4</sup>National Institute for Infectious Diseases L. Spallanzani, IRCCS, Rome, Italy; <sup>5</sup>University Hospital of Siena, Siena, Italy; <sup>6</sup>San Raffaele Scientific Institute, Milan, Italy; <sup>7</sup>University of Bologna, Bologna, Italy; <sup>8</sup>Department of Oncology, University of Milan, Milan, Italy

\*Corresponding author. Department of Experimental Medicine and Surgery, University of Rome Tor Vergata, Via Montpellier 1, 00133 Rome, Italy.

Tel: +39-06-72596566 or 06-72596552; E-mail: ceccherini@med.uniroma2.it

†Present address: GENOMA-Molecular Genetics Laboratory, Rome, Italy.

‡Members are listed in the Acknowledgements section.

Received 10 May 2018; returned 12 June 2018; revised 31 July 2018; accepted 5 August 2018

**Objectives:** We evaluated the association between pre-ART HIV DNA and HIV-infected participant characteristics at baseline as well as with their response to first-line ART.

**Methods:** Four hundred and thirty-three patients from the ICONA cohort, starting first-line ART after the year 2000, were analysed. Pre-ART HIV DNA was quantified with the modified COBAS TaqMan HIV-1 Test and normalized by CD4+ T cells. Linear correlation between pre-ART HIV DNA and other continuous markers (HIV RNA, CD4 count, markers of inflammation and coagulation) at baseline was evaluated by means of Pearson correlation coefficient and a linear regression model. Survival analyses and Cox regression models were used to study the association between pre-ART HIV DNA and time to viro-immunoclinical events.

**Results:** Pre-ART HIV DNA [median (IQR): 10 702 (3397–36 632) copies/10<sup>6</sup> CD4+ T cells] was correlated with pre-ART HIV RNA [ $R^2 = +0.44$ , ( $P < 0.0001$ )], CD4+ T cells [ $R^2 = -0.58$ , ( $P < 0.0001$ )] and CD4/CD8 ratio [ $R^2 = -0.48$ , ( $P < 0.0001$ )], while weaker correlations were observed with CD8+ T cells ( $R^2 = -0.20$ ,  $P = 0.01$ ), IL-6 ( $R^2 = +0.16$ ,  $P = 0.002$ ) and soluble CD14 ( $R^2 = +0.09$ ,  $P = 0.05$ ). Patients with higher pre-ART HIV DNA showed lower rate and delayed virological response (defined as HIV RNA  $\leq 50$  copies/mL), compared with those having lower HIV DNA (67.2% for  $>10\,000$ , 81.1% for 1000–10 000 and 86.4% for 10–1000 copies/10<sup>6</sup> CD4+ T cells;  $P = 0.0004$ ). Higher pre-ART HIV DNA was also correlated with increased risk of virological rebound (defined as HIV RNA  $>50$  copies/mL) by 24 months (17.2% for  $>10\,000$ , 7.4% for 1000–10 000 and 4.3% for 10–1000 copies/10<sup>6</sup> CD4+ T cells;  $P = 0.0048$ ). Adjusted HRs of all virological rebound definitions confirmed these findings ( $P \leq 0.02$ ).

**Conclusions:** Pre-ART HIV DNA, along with HIV RNA and CD4+ T cell count, should be considered as a new staging marker to better identify people at lower (or higher) risk of viral rebound following achievement of virological suppression ( $\leq 50$  copies/mL).

### Introduction

HIV-1 is known to establish persistent infection through different mechanisms, the main one being HIV-1 DNA persistence in infected cells, such as CD4+ T cells that remain the main contributor to viral persistence and the major obstacle for cure strategies.<sup>1–6</sup> Several

markers have been proposed to measure the viral burden in these cells and among these total HIV DNA is a good candidate due to the simplicity, accuracy and reproducibility of its quantification and the small sample required.<sup>7–11</sup> Total HIV DNA includes both integrated

© The Author(s) 2018. Published by Oxford University Press on behalf of the British Society for Antimicrobial Chemotherapy.

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0/>), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact [journals.permissions@oup.com](mailto:journals.permissions@oup.com).

and unintegrated forms of HIV DNA, which can coexist in infected cells and can actively participate in viral transcription, viral replication and pathogenesis.<sup>12,13</sup> In the setting of drug-naïve patients, total HIV DNA has been shown to be a clinically relevant parameter for disease progression,<sup>14–17</sup> with a predictive power for AIDS or death stronger than that of plasma HIV RNA.<sup>16</sup> Recent studies also suggest that total HIV DNA can predict the time and magnitude of viral rebound in treatment-experienced patients after therapy interruption,<sup>17</sup> as well as early virological failure in pretreated patients receiving simplified regimens.<sup>18</sup> In the setting of long-term effective ART, total HIV DNA in CD4+ T cells not only reflects the size of the replication-competent viral load (VL),<sup>19,20</sup> but has also been linked to multiple treatment outcomes, such as therapeutic failure, residual viraemia and immune recovery,<sup>21,22</sup> thus reinforcing the utility of total HIV DNA monitoring in long-term treatment follow-up.

Very limited data are available regarding the prognostic value of pretreatment HIV DNA to predict the chance of virological suppression and of long-term treatment outcome in patients starting their first-line ART regimen.<sup>23–25</sup> For this reason, this study aims to investigate the association between total HIV DNA (quantified by a modified commercial assay at ART initiation) and other pre-ART viro-immunoclinical parameters, as well as its prognostic value to predict subsequent viral response in patients achieving first viral suppression.

## Methods

### Study population

This prospective study includes retrospectively collected samples from patients belonging to the ICONA Foundation Cohort, a multicentre Italian cohort enrolling and prospectively following ART-naïve HIV-infected patients who participated in the original ICONA study.<sup>26</sup> CD4+ T cells and CD8+ T cell counts and VL measurements were performed on average every 4 months (median 3.4, IQR 2.1–6.0); patients were monitored also for other laboratory parameters (e.g. liver and kidney function, lipids), clinical and treatment data. A biobank collecting plasma and blood cell samples at baseline and yearly is also available.

Our analyses were limited to ART-naïve patients who started an ART regimen including at least three antiretroviral drugs after 1 January 2000 and having a clinical and viro-immunological follow-up of at least 1 year. Each patient at ART initiation had available a stored PBMC aliquot and/or whole blood and plasma samples, for HIV DNA and plasma biomarker evaluation, respectively. All patients were also monitored by standard routine visits and evaluated for the development of AIDS, serious non-AIDS events or death. The following diseases were considered serious non-AIDS events: end-stage liver disease, end-stage renal disease, myocardial infarction, sepsis, non-AIDS-defining cancers and severe cardiovascular events.

All patients signed informed consent forms to participate in the ICONA Foundation Study. The research study protocol was approved by local institutional review boards and by the ethics committee of each participating centre.

### Quantification of plasma HIV-1 RNA

Depending on methodologies available during 2000–16, plasma HIV RNA was routinely determined using three different assays at the different participating centres.<sup>27–29</sup> Details are provided in the [Supplementary Methods](#) (see [Supplementary data](#) at JAC Online).

### Quantification of pre-ART HIV DNA

Total HIV DNA was quantified at University of Rome Tor Vergata before ART initiation (time window for HIV DNA collection  $-12\pm 1$  weeks from ART

initiation) starting from a pellet of at least  $1\times 10^6$  PBMCs (range of  $1\times 10^6$  to  $6\times 10^6$ ) for 272 patients, and from 200  $\mu$ L of whole blood for 161 patients, by using a modified version of the commercial COBAS AmpliPrep/COBAS TaqMan HIV-1 Test v2.0, as previously reported.<sup>10</sup> Detailed methodology is provided in the [Supplementary data](#).

### Quantification of plasma biomarkers

We used commercially available ELISAs to quantify plasma levels of IL-6 and soluble CD14 (sCD14) (R&D Systems), according to the manufacturer protocols.

### Statistical analysis

Patients were ranked in three categories according to *a priori* chosen cut-offs in the HIV DNA levels at ART initiation (pre-ART HIV DNA): 10–1000 copies/ $10^6$  CD4+ T cells; 1000–10 000 copies/ $10^6$  CD4+ T cells; and  $>10\,000$  copies/ $10^6$  CD4+ T cells.

Categorical factors at baseline were compared across HIV DNA groups using the  $\chi^2$  test.

### Virological suppression

Standard survival analysis based on Kaplan–Meier estimates was used to evaluate the association between pre-ART HIV DNA levels and the time to achieve virological suppression, defined as the first of two determinations of plasma HIV RNA  $\leq 50$  copies/mL. These analyses were performed on the overall population ( $n = 433$ ).

### Long-term treatment outcome

To evaluate the possible effect of pre-ART HIV DNA levels on the risk of virological rebound, we included in the analysis only participants who had achieved virological suppression  $\leq 50$  copies/mL ( $n = 397$ ). Standard survival analysis based on Kaplan–Meier estimates was used to evaluate the association between pre-ART HIV DNA and the time to achieve the following primary endpoint: virological rebound, defined according to three different HIV RNA cut-off values: (i) two confirmed plasma HIV RNA determinations  $>50$  copies/mL; (ii) two confirmed plasma HIV RNA determinations  $>200$  copies/mL; and (iii) a single plasma HIV RNA  $>1000$  copies/mL.

Secondary endpoints were: (i) an increase in CD4+ T cell count of at least 200 cells/ $\text{mm}^3$  from pre-ART levels; and (ii) AIDS diagnosis, serious non-AIDS events or death. Kaplan–Meier curves were compared by means of the Fleming–Harrington test.

Cox regression models were used to test the prognostic value of pre-ART HIV DNA (fitted in the  $\log_{10}$  scale) to predict long-term treatment outcomes, after controlling for a number of identified potential confounders. A similar analysis was performed to test the impact of pre-ART HIV RNA on the same outcomes.

A detailed description of the statistical analyses used can be found in the [Supplementary data](#).

Statistical analyses were performed using SAS (version 9.4, SAS Institute, Cary, NC, USA).

## Results

### Patient characteristics

Four-hundred and thirty-three patients were included in this analysis. Baseline patient characteristics according to HIV DNA strata are shown in [Table 1](#). The majority were male ( $n = 350$ , 80.8%) and MSM ( $n = 194$ , 44.8%). The median age was 38 years (IQR 33–46 years).

**Table 1.** Main characteristics of overall population

Characteristics	HIV DNA (per 1×10 <sup>6</sup> CD4 cells)			P value	Total (N = 433)
	10–1000 (n = 44)	1000–10 000 (n = 162)	>10 000 (n = 227)		
Gender, n (%)				0.965	
female	8 (18.2)	32 (19.8)	43 (18.9)		83 (19.2)
Mode of HIV transmission, n (%)				0.017	
IVDU	4 (9.1)	14 (8.6)	25 (11.0)		43 (9.9)
homosexual contacts	26 (59.1)	86 (53.1)	82 (36.1)		194 (44.8)
heterosexual contacts	12 (27.3)	53 (32.7)	100 (44.1)		165 (38.1)
other/unknown	2 (4.5)	9 (5.6)	20 (8.8)		31 (7.2)
Ethnicity, n (%)				0.631	
Black	3 (6.8)	6 (3.7)	12 (5.3)		21 (4.8)
Age, years	40 (33–50)	37 (31–43)	39 (33–47)	0.007	38 (33–46)
Viral RNA, log <sub>10</sub> copies/mL	4.26 (3.56–4.67)	4.56 (4.18–5.00)	5.07 (4.71–5.54)	<0.001	4.84 (4.32–5.35)
HIV subtype				0.341	
B	22 (50.0)	74 (45.7)	114 (50.2)		210 (48.5)
non-B	3 (6.8)	13 (8.0)	28 (12.3)		44 (10.2)
unknown	19 (43.2)	75 (46.3)	85 (37.4)		179 (41.3)
Time from seroconversion to sample, months	39 (21–146)	30 (14–55)	48 (15–87)	0.142	35 (15–79)
CD4 count, cells/mm <sup>3</sup>	371 (293–509)	378 (298–484)	196 (78–321)	<0.001	297 (160–408)
CD8 count, cells/mm <sup>3</sup>	998 (692–1188)	952 (710–1274)	841 (557–1160)	0.026	895 (622–1207)
CD4/CD8 count, cells/mm <sup>3</sup>	0.39 (0.35–0.48)	0.41 (0.30–0.56)	0.23 (0.12–0.35)	<0.001	0.33 (0.19–0.46)
IL-6, pg/mL	1.7 (1.2–4.2)	1.4 (0.8–2.5)	1.9 (1.1–3.4)	<0.001	1.7 (1.0–2.9)
sCD14, pg/mL	2.8 (2.2–4.0)	2.7 (1.9–3.3)	2.9 (2.1–4.0)	0.032	2.8 (2.1–3.8)
Year of stored sample	2011 (2010–2012)	2011 (2008–2011)	2010 (2003–2011)	<0.001	2010 (2004–2011)
White blood cells, 1000/mm <sup>3</sup>	5400 (4300–6900)	5250 (4420–5900)	4400 (3300–5685)	<0.001	4990 (3810–5900)
HIV DNA, copies per 1 × 10 <sup>6</sup> CD4 cells	618 (371–786)	4029 (2576–6520)	34 876 (17 692–82 539)	<0.001	10 702 (3397–36 632)
HBsAg, n (%)				0.236	
negative	34 (77.3)	135 (83.3)	195 (85.9)		364 (84.1)
positive	1 (2.3)	0 (0.0)	1 (0.4)		2 (0.5)
not tested	9 (20.5)	27 (16.7)	31 (13.7)		67 (15.5)
CMV, n (%)				0.771	
negative	2 (4.5)	9 (5.6)	14 (6.2)		25 (5.8)
positive	23 (52.3)	76 (46.9)	120 (52.9)		219 (50.6)
not tested	19 (43.2)	77 (47.5)	93 (41.0)		189 (43.6)
HCV Ab, n (%)				0.897	
negative	34 (77.3)	121 (74.7)	178 (78.4)		333 (76.9)
positive	6 (13.6)	24 (14.8)	26 (11.5)		56 (12.9)
not tested	4 (9.1)	17 (10.5)	23 (10.1)		44 (10.2)

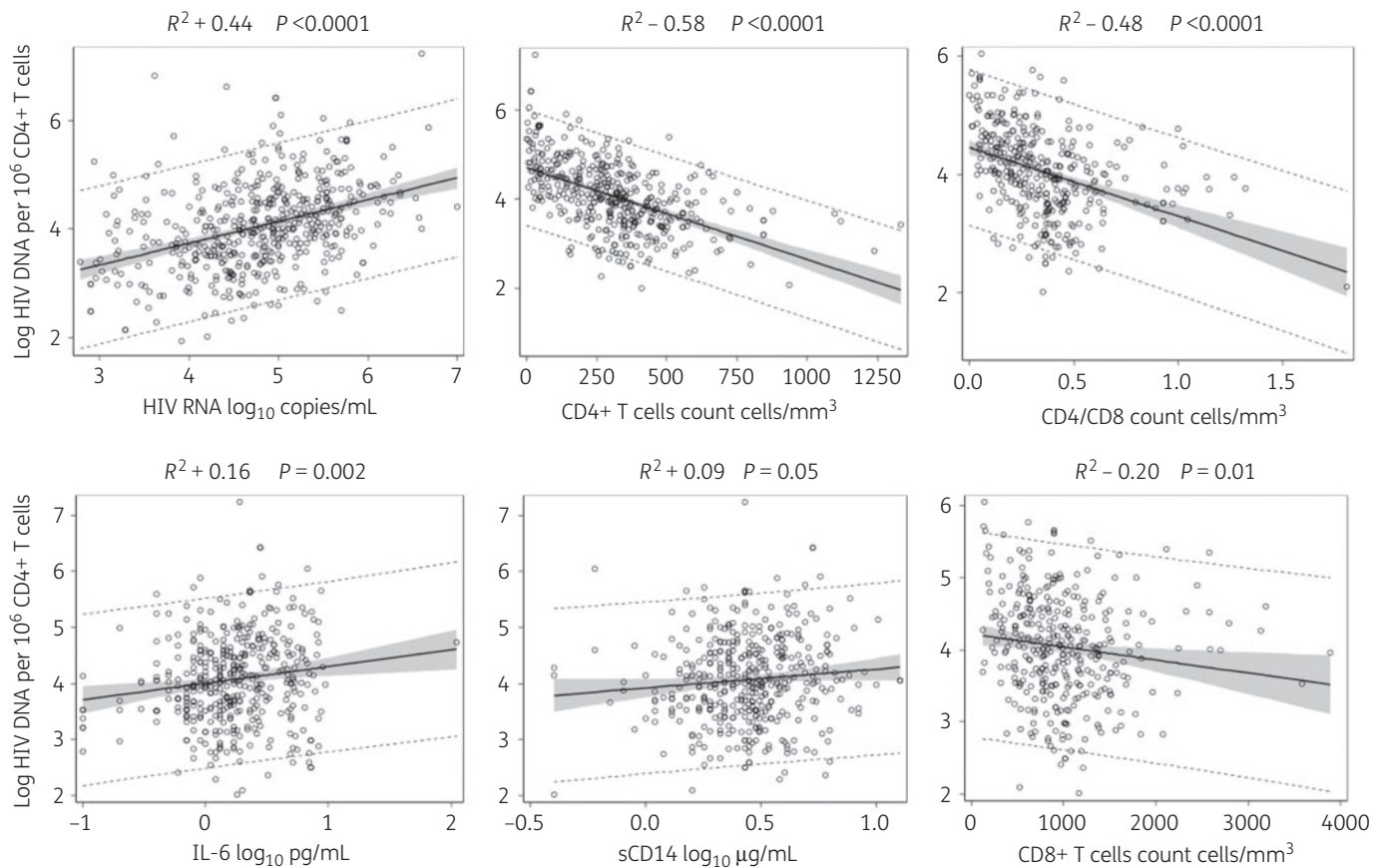
HBsAg, Hepatitis B surface antigen; CMV, Cytomegalovirus. Unless otherwise indicated, results shown are median (IQR).

For a subset of individuals ( $n = 7$ ), for whom both blood samples and PBMCs were available, we performed the total HIV DNA quantification by both sources. Results of HIV DNA quantification normalized for CD4 cells were comparable [median (IQR) HIV DNA was 3.5 (3.2–4.6) log<sub>10</sub> copies/10<sup>6</sup> CD4+ T cells in blood samples and 3.7 (3.4–4.7) log<sub>10</sub> copies/10<sup>6</sup> CD4+ T cells in PBMCs;  $P = 0.13$ ].

As per inclusion criteria, all patients were enrolled before ART initiation and had a median plasma HIV RNA and a median HIV DNA of 4.84 (IQR 4.32–5.35) log<sub>10</sub> copies/mL and 10 702 (IQR 3397–36 632) copies/10<sup>6</sup> CD4+ T cells, respectively. Median CD4+ T cell count was 297 (IQR 160–408) cells/mm<sup>3</sup>. The median (IQR) year of sample collection (corresponding to the start of ART) was

2010 (2004–11). Data regarding ART, time to achieve virological suppression ( $\leq 50$  copies/mL) and genotypic susceptibility score (GSS) of ART are shown in Table S1.

By stratifying patients according to pre-specified clinical cut-offs for pre-ART HIV DNA, more than half of patients ( $n = 227$ , 52.4%) had a total HIV DNA higher than >10 000 copies/10<sup>6</sup> CD4+ T cells, 37.4% ( $n = 162$ ) between 1000 and 10 000, and only 10.2% ( $n = 44$ ) between 10 and 1000 copies/10<sup>6</sup> CD4+ T cells. By correlating pre-ART HIV DNA with other viro-immunological parameters, higher plasma HIV RNA ( $P < 0.0001$ ), lower CD4+ T cell count ( $P < 0.0001$ ) and lower CD4/CD8 ratio ( $P < 0.0001$ ) at baseline were more frequently observed in the patients with pre-ART HIV DNA



**Figure 1.** Linear correlations of pre-ART HIV DNA with viro-immunological parameters at baseline of ART in first-line treated ART patients.

>10 000 copies/ $10^6$  CD4+ T cells than in those with pre-ART HIV DNA in the ranges 1000–10 000 and 10–1000 copies/ $10^6$  CD4+ T cells (Table 1). As expected, correlations between higher levels of pre-ART HIV DNA and (i) higher plasma HIV RNA; (ii) lower CD4+ T cell count; and (iii) lower CD4/CD8 ratio, all collected at baseline of ART, were confirmed by significant linear correlations (Pearson  $R^2$  and  $P$  values: +0.44 and <0.0001; –0.58 and <0.0001; and –0.48 and <0.0001, respectively) (Figure 1). Conversely, less strong correlations were found between HIV DNA and IL-6 ( $P = 0.002$ ), sCD14 ( $P = 0.05$ ) and CD8+ T cells ( $P = 0.01$ ) (Figure 1).

### Role of pre-ART HIV DNA in influencing treatment outcome

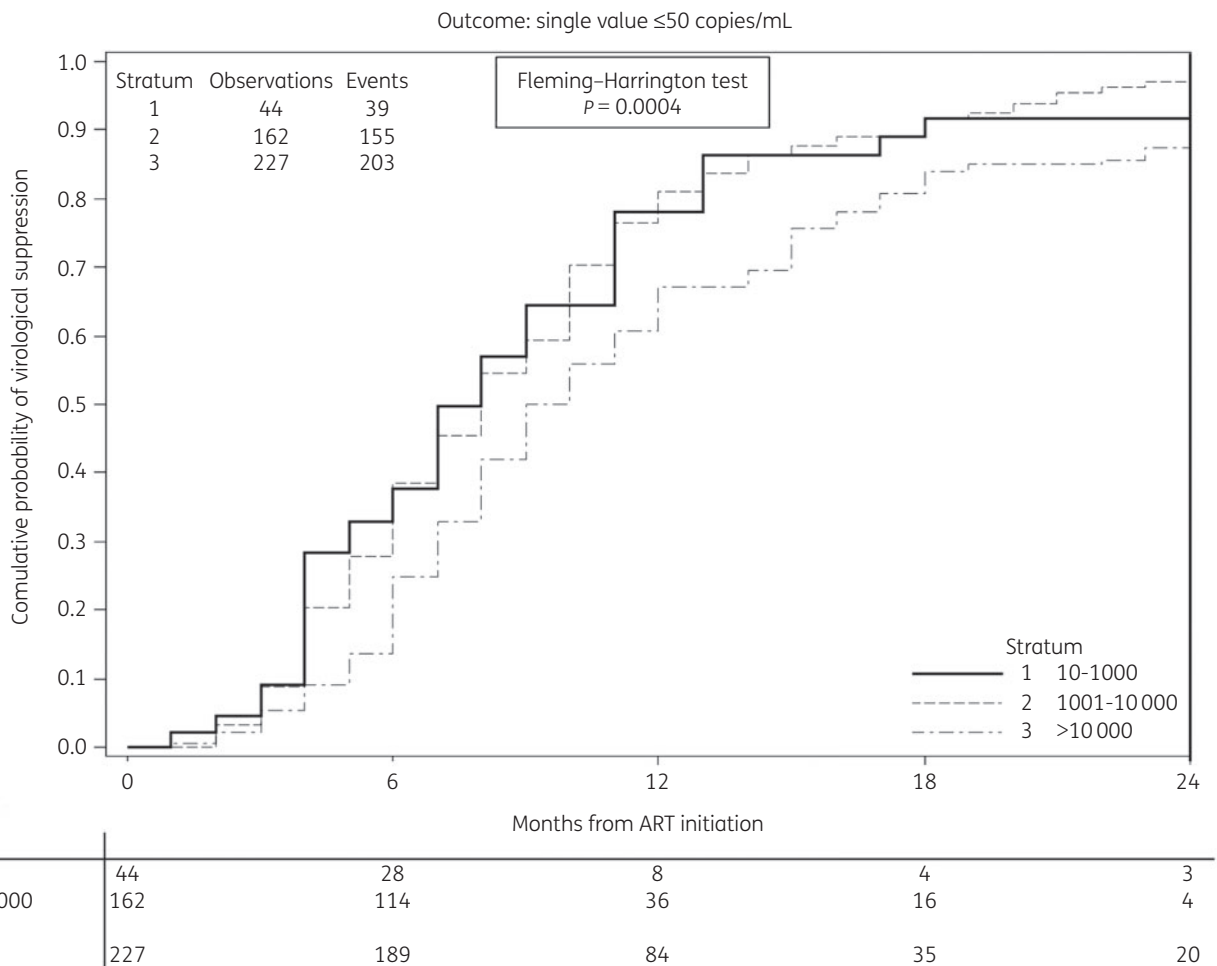
Kaplan–Meier curves and Cox regression multivariable analyses were performed to characterize the association between pre-ART HIV DNA and therapy outcomes in our study population.

#### Association between pre-ART HIV DNA and time to achieve virological suppression $\leq 50$ copies/mL

Of the 433 patients analysed, 397 achieved virological suppression. In particular, the probability of achieving plasma HIV RNA  $\leq 50$  copies/mL by 12 and 24 months after starting ART was 73.6% (95% CI 69.3–77.8) and 92.1% (95% CI 89.4–94.9), respectively.

By stratifying patients according to pre-ART HIV DNA levels, we found that the cumulative probability of virological suppression was significantly lower in the group starting ART with the highest HIV DNA (Figure 2). In particular, the proportion of patients reaching virological suppression was 67.2% (95% CI 60.9–73.5) in the pre-ART HIV DNA >10 000 copies/ $10^6$  CD4+ T cells group, 81.1% (95% CI 75.0–87.3) in the pre-ART HIV DNA 1000–10 000 copies/ $10^6$  CD4+ T cells group and 86.4% (95% CI 75.4–97.3) in the pre-ART HIV DNA 10–1000 copies/ $10^6$  CD4+ T cells group ( $P = 0.0004$  by Fleming–Harrington test). Further, the estimated median time to achieve virological suppression in patients with a pre-ART HIV DNA >10 000 copies/ $10^6$  CD4+ T cells was significantly longer than that observed in the remaining two groups [median time to reach virological suppression: 10.0 (IQR 9–11) months for patients with HIV DNA >10 000 copies/ $10^6$  CD4+ T cells; 8.0 (IQR 7–9) months for patients with 1000–10 000 copies/ $10^6$  CD4+ T cells; and 8.0 (IQR 6–9) months for patients with 10–1000 copies/ $10^6$  CD4+ T cells] (Figure 2).

Overall, by 6 months of treatment the percentage of patients with HIV RNA  $\leq 50$  copies/mL was low, probably due to a large proportion of patients starting treatment with less potent ART. At 6 and 12 months there was a statistically significant difference in the percentage of patients who were virologically suppressed according to the three different HIV DNA strata [patients with plasma HIV RNA  $\leq 50$  copies/mL at 6 months after starting ART



**Figure 2.** Kaplan-Meier probability estimates of achieving plasma HIV-1 RNA  $\leq 50$  copies/mL. Statistically significant difference was assessed by the Fleming-Harrington test. Analysis was performed on 433 patients.

were 18/42 (43%), 53/153 (35%) and 32/215 (15%), and at 12 months were 31/36 (86%), 127/142 (89%) and 137/198 (69%), respectively, for baseline HIV DNA of 10–1000, 1000–10 000 and >10 000 copies/ $10^6$  CD4+ T cells; false discovery rate (FDR)  $P < 0.0001$  at both timepoints].

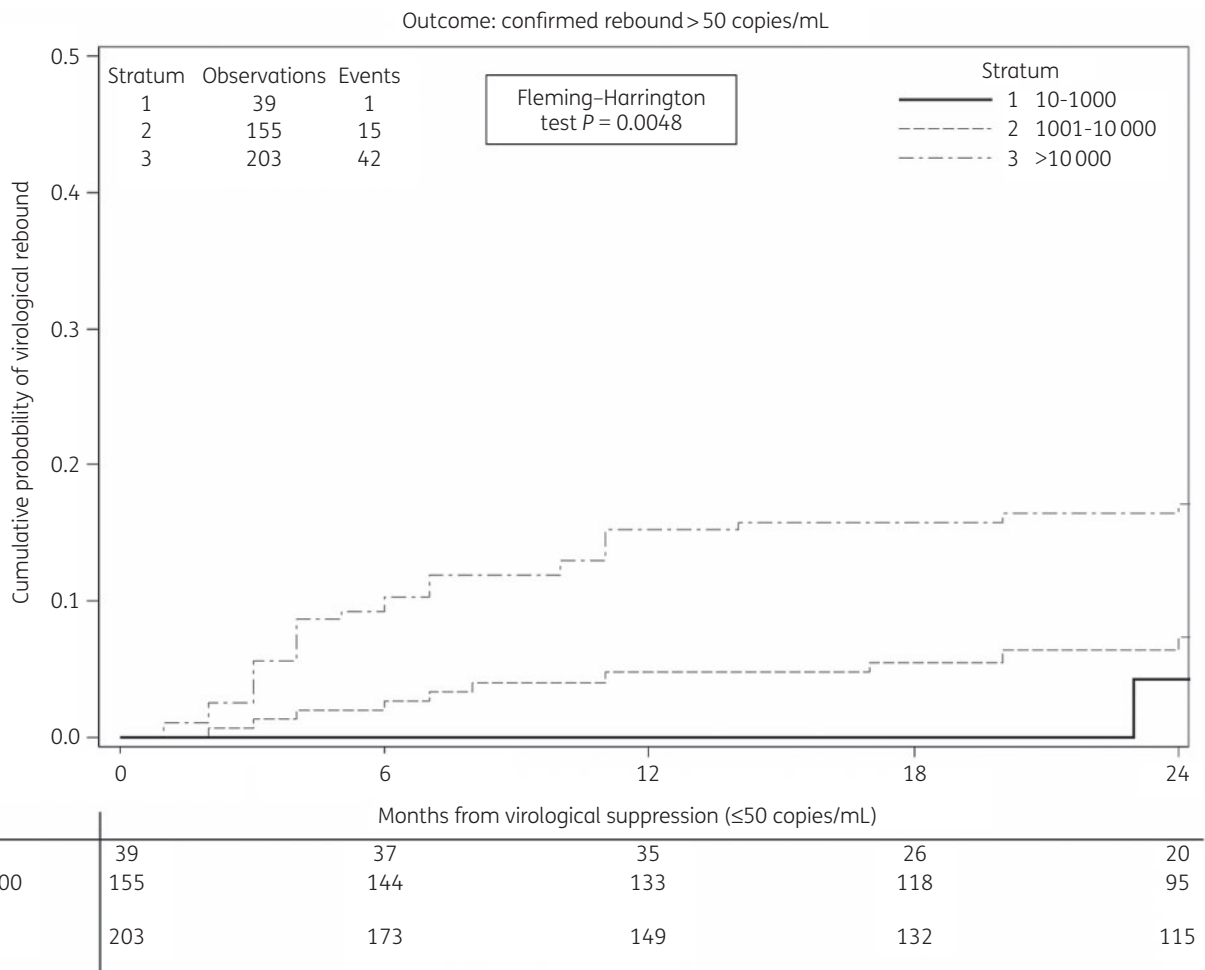
#### Association of pre-ART HIV DNA with the virological rebound

By considering the 397 patients achieving virological suppression, the probability of experiencing virological rebound, defined by two confirmed plasma HIV RNA determinations  $> 50$  copies/mL, was 12% (95% CI 8.6–15.5). By stratifying patients for the three different pre-ART HIV DNA levels, increasing rates of virological rebound were found with increasing pre-ART HIV DNA ( $P = 0.0048$  by Fleming-Harrington test) (Figure 3). In particular, by 12 and 24 months the proportion of patients experiencing virological rebound for those with a pre-ART HIV DNA  $> 10 000$  copies/ $10^6$  CD4+ T cells was significantly higher than that observed in the remaining two patient categories [15.2% (95% CI 10.1–20.3) by 12 months and 17.2% (95% CI 11.7–22.6) by 24 months for patients with HIV

DNA  $> 10 000$  copies/ $10^6$  CD4+ T cells; 4.8% (95% CI 1.3–8.2) by 12 months and 7.4% (95% CI 2.9–11.8) by 24 months for patients with 1000–10 000 copies/ $10^6$  CD4+ T cells; and 0.0% (95% CI 0.0–0.0) by 12 months and 4.3% (95% CI: 0–12.7) by 24 months for those with 10–1000 copies/ $10^6$  CD4+ T cells]. The results were similar using different definitions of virological rebound, such as two confirmed plasma HIV RNA determinations  $> 200$  copies/mL or a single HIV RNA determination  $> 1000$  copies/mL, even if the  $P$  values by Fleming-Harrington tests were not significant ( $P = 0.20$  and  $P = 0.16$ , respectively; data not shown).

#### Association of pre-ART HIV DNA with increase in CD4+ T cell count and with clinical events

From ART initiation to follow-up, 83.4% (95% CI 79.4–87.4) of the 433 individuals increased their CD4+ T cells by at least 200 cells/ $\text{mm}^3$  and 2.7% (95% CI 1.1–4.3) of patients experienced an AIDS diagnosis, serious non-AIDS event or death. The survival analyses by the Kaplan-Meier curves showed that there was no evidence for a difference in the rates of CD4+ T cell gain and in the risk of AIDS diagnosis, serious non-AIDS events or death by



**Figure 3.** Kaplan-Meier probability estimates of achieving virological rebound, defined as two confirmed plasma HIV RNA determinations >50 copies/mL, after the virological suppression. Statistically significant difference was assessed by the Fleming-Harrington test. Analysis was performed on 397 patients.

increasing levels of pre-ART HIV DNA ( $P = 0.95$  and  $P = 0.11$ , by Fleming-Harrington test, respectively; data not shown).

### Relative prognostic value of pre-ART HIV DNA and HIV RNA to predict treatment outcome

In order to directly compare the relative prognostic value of pre-ART HIV DNA and pre-ART HIV RNA for virological suppression and long-term virological rebound, first we created a bivariable Cox regression model including only these two factors, both fitted to the  $\log_{10}$  scale (Table 2). We then proceeded to perform the comparison after controlling for potential confounding factors (three separate sets of confounders) (Table 3).

#### Prognostic value of pre-ART HIV DNA and pre-ART HIV RNA on the chance of virological suppression and risk of developing clinical events

By the initial bivariable analysis, higher pre-ART HIV DNA and higher pre-ART HIV RNA were both associated with a lower hazard of virological suppression, the magnitude of the effect being

slightly higher for HIV DNA [HR per  $\log_{10}$  increase 0.84 (95% CI 0.72–0.97) and 0.74 (0.64–0.85), respectively] (Table 2). However, after controlling for other confounding factors, only pre-ART HIV RNA remained independently associated with the probability of virological suppression ( $P < 0.0001$  in both models A and B) and weakly associated with the CD4+ T cell count recovery (raw  $P$  value = 0.028 in model A and 0.011 in model B) (Table 3).

Of note, HIV DNA was associated with the risk of AIDS diagnosis or the occurrence of a serious non-AIDS event or death after controlling for HIV RNA alone (Table 2), but neither viral parameter was associated with this outcome after further adjusting for the other factors (Table 3).

#### Prognostic value of pre-ART HIV DNA and pre-ART HIV RNA on virological rebound

Looking at the risk of virological rebound in people who initially achieved virological suppression, in the bivariable model, only patients with a higher pre-ART HIV DNA were at higher risk of virological rebound, for all definitions of rebound (confirmed >50 copies/mL, confirmed >200 copies/mL or single >1000 copies/mL),

**Table 2.** Relative prognostic value of 1 log<sub>10</sub> higher pre-ART HIV DNA and pre-ART HIV RNA in uni- and bivariable Cox analyses

	HRs associated with 1 log <sub>10</sub> increase in the markers from fitting a Cox regression model			
	pre-ART HIV DNA		pre-ART HIV RNA	
	unadjusted	mutually adjusted	unadjusted	mutually adjusted
VL ≤50 copies/mL	0.76 (0.66–0.87)	0.84 (0.72–0.97)	0.70 (0.61–0.80)	0.74 (0.64–0.85)
raw <i>P</i> value	<0.0001	0.018	<0.0001	<0.0001
FDR-adjusted <i>P</i> value	0.0006	0.033	0.0006	0.0006
CD4+ T cell count gain >200 cells/mm <sup>3</sup> above pre-ART	0.93 (0.81–1.07)	0.87 (0.75–1.02)	1.11 (0.96–1.28)	1.17 (1.00–1.36)
raw <i>P</i> value	0.325	0.077	0.154	0.046
FDR-adjusted <i>P</i> value	0.371	0.123	0.231	0.079
VL rebound				
confirmed >50 copies/mL	2.20 (1.54–3.15)	1.96 (1.32–2.92)	1.66 (1.12–2.47)	1.27 (0.84–1.93)
raw <i>P</i> value	<0.0001	0.0008	0.012	0.258
FDR-adjusted <i>P</i> value	0.0006	0.003	0.024	0.310
confirmed >200 copies/mL	2.42 (1.37–4.29)	2.94 (1.58–5.47)	0.98 (0.56–1.73)	0.67 (0.37–1.21)
raw <i>P</i> value	0.002	0.0007	0.948	0.184
FDR-adjusted <i>P</i> value	0.006	0.003	0.948	0.245
single value >1000 copies/mL	1.93 (1.20–3.08)	2.24 (1.36–3.70)	0.93 (0.60–1.45)	0.73 (0.46–1.14)
raw <i>P</i> value	0.006	0.002	0.750	0.168
FDR-adjusted <i>P</i> value	0.013	0.006	0.818	0.237
AIDS, serious non-AIDS events or death	2.70 (1.41–5.17)	2.79 (1.36–5.74)	1.51 (0.78–2.92)	0.97 (0.48–1.95)
raw <i>P</i> value	0.003	0.005	0.221	0.927
FDR-adjusted <i>P</i> value	0.008	0.012	0.279	0.948

independent of pre-ART HIV RNA [HR 1.96 (95% CI 1.32–2.92); 2.94 (95% CI 1.58–5.47); and 2.24 (95% CI 1.36–3.70), respectively]. In contrast, after controlling for HIV DNA, pre-ART HIV RNA was not associated with the risk of viral rebound whichever definition was used (Table 2). Interestingly, in all three models, with adjustment for other confounding factors, higher pre-ART HIV DNA was confirmed to be the only factor to have a strong prognostic value for viral rebound, whichever definition was applied for it (Table 3). In particular, when virological rebound was defined as a single determination of plasma HIV RNA >1000 copies/mL or two confirmed plasma determinations of HIV RNA >200 copies/mL, the risk that this event occurred in patients with a pre-ART HIV DNA >1000 copies/10<sup>6</sup> CD4+ T cells was more than 2-fold or even 4-fold higher (according to these two virological rebound definitions) than in patients with a pre-ART HIV DNA <1000 copies/10<sup>6</sup> CD4+ T cells. Interestingly, this association was also found using the third model with final adjustment for antiretrovirals being stopped prior to achieving VL ≤50 copies/mL, time to achieve VL ≤50 copies/mL, total number of drugs used prior to VL ≤50 copies/mL and the GSS (Table 3).

## Discussion

In this study, we performed a comprehensive evaluation of the role of pre-ART HIV DNA, defined as the total HIV DNA normalized by CD4+ T cell count, in the treatment outcome of patients starting first-line ART. The most important finding of our study is that in people who originally achieved viral suppression ≤50 copies/mL, pre-ART HIV DNA level was a strong and significant predictor of virological rebound. Indeed, Cox multivariable estimates

confirmed that pre-ART HIV DNA/10<sup>6</sup> CD4+ T cells, and not pre-ART HIV RNA, was an independent factor associated with faster virological rebound: 1 log<sub>10</sub> HIV DNA higher resulted in a 4-fold higher risk of virological rebound, with an HIV RNA threshold of 200 copies/mL.

So far the predictive value of total HIV DNA in treatment outcomes, such as virological rebound, immune recovery and cell activation status, and also in the setting of de-escalation, has been evaluated in extensively ART-pretreated populations.<sup>7,17–22,31</sup>

Few studies have clearly defined the role of total HIV DNA when measured in drug-naïve patients before first ART initiation. Previous papers have mainly focused on heterogeneous populations typically of small sample size.<sup>23–25,32</sup> For example, a prospective multicentre study has tried to assess the predictive value of PBMC HIV-1 DNA for determining virological and immunological outcomes in a cohort of 148 patients who were treated (starting from 1998) with a first-line PI-containing regimen. This analysis showed that a higher baseline HIV-1 DNA level was associated with an increased risk of virological failure after 1 year of ART, independently of other factors considered.<sup>23</sup> The Monark study showed that, analysing baseline HIV DNA in whole blood using real-time PCR in 46 antiretroviral-naïve patients receiving lopinavir/ritonavir monotherapy, the non-responders (with HIV RNA >50 copies/mL) at 48 weeks had significantly higher baseline HIV-1 DNA load than responders (HIV RNA ≤50 copies/mL).<sup>24</sup> In our analysis, we found a direct association between pre-ART total HIV DNA and the risk of virological rebound, after virological suppression during therapy, in a large cohort of patients starting ART. Moreover, this association was independent of pre-ART plasma HIV RNA and CD4+ T cell count, suggesting that HIV DNA contains information related to

**Table 3.** Relative prognostic value of 1 log<sub>10</sub> higher pre-ART HIV DNA and pre-ART HIV RNA in multivariable Cox analyses

Characteristic	HRs associated with 1 log <sub>10</sub> increase in the markers from fitting a Cox regression model					
	HIV DNA			HIV RNA		
	adjusted <sup>a</sup>	adjusted <sup>b</sup>	adjusted <sup>c</sup>	adjusted <sup>a</sup>	adjusted <sup>b</sup>	adjusted <sup>c</sup>
VL ≤50 copies/mL	0.98 (0.82–1.16)	0.96 (0.80–1.15)		0.74 (0.66–0.83)	0.75 (0.67–0.85)	
raw P value	0.796	0.674		<0.001	<0.001	
FDR-adjusted P value	0.839	0.749		0.004	0.004	
CD4 count gain >200 cells/mm <sup>3</sup> above pre-ART	0.91 (0.76–1.08)	0.88 (0.73–1.05)		1.15 (1.01–1.30)	1.17 (1.04–1.33)	
raw P value	0.282	0.164		0.028	0.011	
FDR-adjusted P value	0.368	0.289		0.065	0.03	
VL rebound						
confirmed >50 copies/mL	1.76 (1.09–2.82)	1.95 (1.18–3.22)	1.94 (1.16–3.25)	1.25 (0.89–1.76)	1.13 (0.80–1.61)	1.19 (0.81–1.75)
raw P value	0.020	0.009	0.011	0.198	0.492	0.386
FDR-adjusted P value	0.05	0.03	0.03	0.312	0.568	0.463
confirmed >200 copies/mL	4.05 (1.82–9.02)	5.65 (2.37–13.49)	4.88 (2.01–11.83)	0.79 (0.52–1.21)	0.72 (0.45–1.14)	0.75 (0.45–1.24)
raw P value	<0.001	<0.001	<0.001	0.280	0.161	0.259
FDR-adjusted P value	0.0043	0.0043	0.0043	0.368	0.289	0.368
single value >1000 copies/mL	2.56 (1.33–4.92)	4.01 (1.92–8.39)	3.75 (1.78–7.93)	0.79 (0.56–1.12)	0.74 (0.51–1.09)	0.72 (0.47–1.12)
raw P value	0.005	<0.001	<0.001	0.182	0.133	0.142
FDR-adjusted P value	0.019	0.0043	0.0043	0.303	0.284	0.284
AIDS, serious non-AIDS events or death	1.60 (0.66–3.89)	1.85 (0.71–4.80)		0.99 (0.54–1.82)	0.92 (0.45–1.88)	
raw P value	0.297	0.208		0.986	0.811	
FDR-adjusted P value	0.371	0.312		0.986	0.839	

<sup>a</sup>Adjusted for calendar year of ART, type of regimen started, CD4 count and RNA/DNA at ART.

<sup>b</sup>Adjusted for calendar year of ART, type of regimen started, CD4 count, RNA/DNA at ART, age, smoking, HCV status, IL-6 and sCD14.

<sup>c</sup>Adjusted for calendar year of ART, type of regimen started, CD4 count, RNA/DNA at ART, age, smoking, HCV status, whether person had stopped anti-retrovirals prior to achieving VL ≤ 50 copies/mL, time to achieve VL ≤ 50 copies/mL, total number of drugs used prior to baseline and HIV database GSS of baseline regimen.

the patient's total viral burden that has prognostic value over and above what is currently used to calculate patient failure risk score in clinical practice. A similar finding, although for a much smaller cohort, had previously been reported by Hatzakis *et al.*<sup>25</sup> In that paper, cellular HIV-1 DNA load was the only parameter associated with the chance of achieving sustained virological response in people starting treatment from being ART-naive. In other words, our analysis confirms the role of pre-ART HIV RNA to predict initial virological response to treatment,<sup>17,33</sup> while diminishing its role as a predictor of viral rebound, once HIV DNA is accounted for.

On the basis of our results, the quantification of HIV DNA levels in CD4+ T cells before therapy should be considered, besides what is currently used in the clinics, to help identify people at lower (or higher) risk of viral rebound following achievement of suppression. Our data also provide indirect evidence to guide the choice of first treatment in ART-naive individuals.

For instance, it is known that, compared with other available drug classes, the inclusion of an integrase strand transfer inhibitor (INSTI) in first-line combinations is associated with faster HIV-1 VL decay<sup>34</sup> and a larger reduction of total HIV-1 DNA in virologically suppressed HIV-1-infected patients.<sup>35</sup> On the basis of our results it could be argued that the use of an INSTI is appropriate in patients with high baseline levels of HIV DNA and that their use may have long-term benefits by reducing HIV DNA in people with controlled HIV RNA.

Interestingly, higher levels of pre-ART HIV DNA showed a good correlation with higher levels of several inflammatory markers associated with T cell immune activation, such as IL-6, sCD14 and CD8+ T cells, supporting the association between HIV DNA and systemic inflammation, and thus clinical progression. These results are, however, in contrast with a recent paper published by Gandhi *et al.*,<sup>36</sup> showing that levels of HIV DNA (pre-ART and during therapy) are not associated with different levels of inflammatory biomarkers such as IL-6, sCD14 and sCD163. Because of these conflicting findings, studies including higher numbers of patients will be necessary to estimate the real role of HIV DNA in the inflammatory process and thus to better understand the role of this parameter in the definition of the inflammatory process and thus clinical progression.

In addition, it needs to be noted that, to date, the major problem with measuring HIV DNA is the lack of reproducibility of different tests and different samples used for the analysis. Currently, there are different in-house protocols to quantify HIV DNA starting from CD4+ T cells, PBMC or whole blood, by measuring total HIV DNA, proviral-integrated or two long terminal repeats (2 LTRs) and few commercial assays (so far there is a commercial kit 'Generic HIV DNA Cell' from Biocentric, Bandol, France, largely used in France, and another recently developed from Diatheva, HIV-1 DNA qPCR kit, for research use only). For our analysis we used a modified



commercial assay measuring both integrated, long-lived proviral DNA and unintegrated short-lived HIV DNA.<sup>10</sup> Unlike the other assays described in this paragraph, this assay is simple and may be routinely applied, because it is already largely used in clinical practice for HIV RNA measurement all over the world.

A possible limitation of our analysis is the lack of adherence data, which is a known determinant of virological response to ART, particularly in the presence of larger reservoir sizes. On the other hand, it is unlikely that adherence level is associated with HIV DNA burden in individual patients before starting ART. Of note, the analysis of the risk of viral rebound is restricted to people who did achieve viral suppression  $\leq 50$  copies/mL on ART. When we compared the characteristics of people who ever achieved HIV RNA  $\leq 50$  copies/mL (included in the time to rebound analysis) and those who never achieved viral suppression (excluded), we found that included patients were more likely to be hepatitis-negative, MSM and of Italian origin (Table S2). These data confirm that patients evaluated for time to viral rebound analysis were a selected population of participants who are more likely to be adherent to treatment. Therefore, selection bias cannot be ruled out and it needs to be acknowledged that the results of our main analysis are only applicable to populations presumed to have good adherence to treatment.

In conclusion, pre-ART HIV DNA showed strong clinical relevance as a marker of HIV burden and reservoir and, although it is unable to distinguish between replication-competent and replication-defective viral forms, it appears to be useful to predict the course of the infection and also under-exposure to ART. Measurement of HIV DNA while a person is still untreated should be considered both to inform treatment choices and help predict the long-term efficacy of ART. Moreover, in the absence of other sensitive and reproducible tests, because the assay used in our analysis is simple, rapid and easily obtainable on the market, it stands as a promising candidate for standardization and measurement of the HIV reservoir in clinical practice.

## Acknowledgements

Some of these data were presented at the annual Conference on Retroviruses and Opportunistic Infections (CROI) 2016, Boston, 22–25 February 2016 (Abstract number 946).

We thank Roche Diagnostics Italy for providing reagents and Dr Mattia Campus for technical assistance on HIV DNA analysis and all members of the Icona Foundation Study Group.

## Members of the ICONA Foundation Study Group

Board of directors: A. d'Arminio Monforte (President), A. Antinori, A. Castagna, F. Castelli, R. Cauda, G. Di Perri, M. Galli, R. Iardino, G. Ippolito, A. Lazzarin, G. C. Marchetti, C. F. Perno, G. Rezza, F. von Schloesser and P. Viale. Scientific secretaries: A. d'Arminio Monforte, A. Antinori, A. Castagna, F. Ceccherini-Silberstein, A. Cozzi-Lepri, E. Girardi, S. Lo Caputo, C. Mussini, M. Puoti and C. F. Perno. Steering committee: M. Andreoni, A. Ammassari, A. Antinori, C. Balotta, A. Bandera, P. Bonfanti, S. Bonora, M. Borderi, A. Calcagno, L. Calza, M. R. Capobianchi, A. Castagna, F. Ceccherini-Silberstein, A. Cingolani, P. Cinque, A. Cozzi-Lepri, A. d'Arminio Monforte, A. De Luca, A. Di Biagio, E. Girardi, N. Gianotti, A. Gori, G. Guaraldi, G. Lapadula, M. Lichtner, S. Lo Caputo, G. Madeddu, F. Maggiolo, G. Marchetti, S. Marcotullio, L. Monno, C. Mussini, S. Nozza, M. Puoti, E. Quiros Roldan, R. Rossotti, S. Rusconi, M. M. Santoro, A. Saracino and M.

Zaccarelli. Statistical and monitoring team: A. Cozzi-Lepri, I. Fanti, L. Galli, P. Lorenzini, A. Rodano, M. Shanyinde and A. Tavelli. Biological Bank INMI: F. Carletti, S. Carrara, A. Di Caro, S. Graziano, F. Petrone, G. Prota, S. Quartu and S. Truffa. Participating physicians and centres: A. Giacometti, A. Costantini and V. Barocci (Ancona); G. Angarano, L. Monno and C. Santoro (Bari); F. Maggiolo and C. Suardi (Bergamo); P. Viale, V. Donati and G. Verucchi (Bologna); F. Castelli, C. Minardi and E. Quiros Roldan (Brescia); T. Quirino and C. Abeli (Busto Arsizio); P. E. Manconi and P. Piano (Cagliari); B. Cacopardo and B. Celesia (Catania); J. Vecchiet and K. Falasca (Chieti); L. Sighinolfi and D. Segala (Ferrara); P. Blanc and F. Vichi (Firenze); G. Cassola, C. Viscoli, A. Alessandrini, N. Bobbio and G. Mazzarello (Genova); C. Mastroianni and I. Pozzetto (Latina); P. Bonfanti and C. Molteni (Lecco); A. Chiopera and P. Milini (Macerata); G. Nunnari and G. Pellicanò (Messina); A. d'Arminio Monforte, M. Galli, A. Lazzarin, G. Rizzardini, M. Puoti, A. Castagna, G. Marchetti, M. C. Moiola, R. Piolini, A. L. Ridolfo, S. Salpietro and C. Tincati, (Milano); C. Mussini and C. Puzzolante (Modena); A. Gori and G. Lapadula (Monza); A. Chirianni, G. Borgia, V. Esposito, R. Orlando, G. Bonadies, F. Di Martino, I. Gentile and L. Maddaloni (Napoli); A. M. Cattelan and S. Marinello (Padova); A. Cascio and C. Colomba (Palermo); F. Baldelli and E. Schiaroli (Perugia); G. Parruti and F. Sozio (Pescara); G. Magnani and M. A. Ursitti (Reggio Emilia); M. Andreoni, A. Antinori, R. Acinapura, G. Baldin, M. Capozzi, R. Cauda, S. Cicalini, A. Cingolani, A. Cristaudo, L. Fontanelli Sulekova, G. Iaiani, A. Latini, I. Mastroianni, M. M. Plazzi, S. Savinelli, A. Vergori, V. Vullo (Roma); M. Cecchetto and F. Viviani (Rovigo); G. Madeddu and P. Bagella (Sassari); A. De Luca and B. Rossetti (Siena); A. Franco and R. Fontana Del Vecchio (Siracusa); D. Francisci and C. Di Giulii (Terni); P. Caramello, G. Di Perri, S. Bonora, G. C. Orofino and M. Sciandra (Torino); M. Bassetti and A. Londero (Udine); G. Pellizzer and V. Manfrin (Vicenza); and G. Starnini and A. Ialungo (Viterbo).

## Funding

This work was supported by the Italian Ministry of Instruction, University and Research (MIUR) (Bandiera InterOmics Protocollo PB05 1°), Aviralia Foundation, Italian Ministry of Health Ricerca Corrente, and by an unconditional contribution of Roche Diagnostics Italy. Icona Foundation is supported by unrestricted grants from Bristol-Myers Squibb, Gilead, Janssen, MSM and Viiv Italy. All funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

## Transparency declarations

The authors have no competing interests that might be perceived to influence the results and/or discussion reported in this paper. However, F. C.-S. reports personal fees from Gilead Sciences, Bristol-Myers Squibb, Abbvie, Roche Diagnostics, Janssen-Cilag, Abbott Molecular, Viiv Healthcare; grants and personal fees from Merck Sharp & Dohme; grants from Italian Ministry of Instruction, University and Research (MIUR). Carlo Federico Perno reports grants from Italian Ministry of Instruction, University and Research (MIUR), and from Aviralia Foundation; personal fees from Gilead Sciences, Abbvie, Roche Diagnostics, Janssen-Cilag, Abbott Molecular; and grants and personal fees from Bristol-Myers Squibb, Merck Sharp & Dohme, and Viiv Healthcare. All other authors have none to declare.

## Author contributions

All authors were involved in study concept discussion, data supervision and manuscript revision. F. C.-S., A. C. L., C. A., E. M., M. R. C. G. M. and A. D. M.: data discussion and interpretation; finalized the draft of the

manuscript. M. S. and E. M.: performed HIV DNA and ELISA analysis, respectively. A. C. L. performed the statistical analysis.

## Supplementary data

Supplementary Methods and Tables S1 and S2 appear as Supplementary data at JAC Online.

## References

- Palmer S, Maldarelli F, Wiegand A *et al.* Low-level viremia persists for at least 7 years in patients on suppressive antiretroviral therapy. *Proc Natl Acad Sci USA* 2008; **105**: 3879–84.
- Murray JM, Zaunders JJ, McBride KL *et al.*; PINT Study Team. HIV DNA sub-species persist in both activated and resting memory CD4+ T cells during antiretroviral therapy. *J Virol* 2014; **88**: 3516–26.
- Murray AJ, Kwon KJ, Farber DL *et al.* The latent reservoir for HIV-1: how immunologic memory and clonal expansion contribute to HIV-1 persistence. *J Immunol* 2016; **197**: 407–17.
- Clutton G, Xu Y, Baldoni PL *et al.* The differential short- and long-term effects of HIV-1 latency-reversing agents on T cell function. *Sci Rep* 2016; **6**: 30749.
- Banga R, Procopio FA, Cavassini M *et al.* *In vitro* reactivation of replication-competent and infectious HIV-1 by histone deacetylase inhibitors. *J Virol* 2015; **90**: 1858–71.
- Gandhi RT, Bosch RJ, Aga E *et al.*; ACTG A5173 team. Residual plasma viraemia and infectious HIV-1 recovery from resting memory CD4 cells in patients on antiretroviral therapy: results from ACTG A5173. *Antivir Ther* 2013; **18**: 607–13.
- Avettand-Fènoël V, Hocqueloux L, Ghosn J *et al.* Total HIV-1 DNA, a marker of viral reservoir dynamics with clinical implications. *Clin Microbiol Rev* 2016; **29**: 859–80.
- Svicher V, Marchetti G, Ammassari A *et al.*; Impact Study Group. Novelities in evaluation and monitoring of human immunodeficiency virus-1 infection: is standard virological suppression enough for measuring antiretroviral treatment success? *AIDS Rev* 2017; **19**: 119–33.
- Lillo FB, Grasso MA, Lodini S *et al.* Few modifications of the Cobas Amplicor HIV Monitor 1.5 test allow reliable quantitation of HIV-1 proviral load in peripheral blood mononuclear cells. *J Virol Methods* 2004; **120**: 201–5.
- Surdo M, Bertoli A, Colucci G *et al.* Total HIV-1 DNA detection and quantification in peripheral blood by COBAS<sup>®</sup> AmpliPrep/COBAS<sup>®</sup> TaqMan<sup>®</sup> HIV-1 Test, v2.0. *Clin Chem Lab Med* 2016; **54**: e57–9.
- De Rossi A, Zanchetta M, Vitone F *et al.* Quantitative HIV-1 proviral DNA detection: a multicentre analysis. *New Microbiol* 2010; **33**: 293–302.
- Chan CN, Trinité B, Lee CS *et al.* HIV-1 latency and virus production from unintegrated genomes following direct infection of resting CD4 T cells. *Retrovirology* 2016; **13**: 1.
- Ho YC, Shan L, Hosmane NN *et al.* Replication-competent noninduced proviruses in the latent reservoir increase barrier to HIV-1 cure. *Cell* 2013; **155**: 540–51.
- Goujard C, Bonarek M, Meyer L *et al.* CD4 cell count and HIV DNA level are independent predictors of disease progression after primary HIV type 1 infection in untreated patients. *Clin Infect Dis* 2006; **42**: 709–15.
- 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**: 366–73.
- Kostrikis LG, Touloumi G, Karanickolas R *et al.* Quantitation of human immunodeficiency virus type 1 DNA forms with the second template switch in peripheral blood cells predicts disease progression independently of plasma RNA load. *J Virol* 2002; **76**: 10099–108.
- Williams JP, Hurst J, Stöhr W *et al.* HIV-1 DNA predicts disease progression and post-treatment virological control. *eLife* 2014; **3**: e03821.
- Torres-Cornejo A, Benmarzouk-Hidalgo OJ, Gutiérrez-Valencia A *et al.* Cellular HIV reservoir replenishment is not affected by blip or intermittent viremia episodes during darunavir/ritonavir monotherapy. *AIDS* 2014; **28**: 201–8.
- Buzon MJ, Martin-Gayo E, Pereyra F *et al.* Long-term antiretroviral treatment initiated at primary HIV-1 infection affects the size, composition, and decay kinetics of the reservoir of HIV-1-infected CD4 T cells. *J Virol* 2014; **88**: 10056–65.
- Kiselinova M, De Spiegelaere W, Buzon MJ *et al.* Integrated and total HIV-1 DNA predict *ex vivo* viral outgrowth. *PLoS Pathog* 2016; **12**: e1005472.
- Rodríguez-Sáinz C, Ramos R, Valor L *et al.* Prognostic value of peripheral blood mononuclear cell associated HIV-1 DNA for virological outcome in asymptomatic HIV-1 chronic infection. *J Clin Virol* 2010; **48**: 168–72.
- Chun TW, Murray D, Justement JS *et al.* Relationship between residual plasma viremia and the size of HIV proviral DNA reservoirs in infected individuals receiving effective antiretroviral therapy. *J Infect Dis* 2011; **204**: 135–8.
- Masquelier B, Taieb A, Reigadas S *et al.*; APROCO-COPILOTE study group. Cellular HIV-1 DNA quantification and short-term and long-term response to antiretroviral therapy. *J Antimicrob Chemother* 2011; **66**: 1582–9.
- Avettand-Fènoël V, Flandre P, Chaix ML *et al.*; MONARK study group. Impact of 48 week lopinavir/ritonavir monotherapy on blood cell-associated HIV-1-DNA in the MONARK trial. *J Antimicrob Chemother* 2010; **65**: 1005–7.
- Hatzakis AE, Touloumi G, Pantazis N *et al.* Cellular HIV-1 DNA load predicts HIV RNA rebound and the outcome of highly active antiretroviral therapy. *AIDS* 2004; **18**: 2261–7.
- d'Arminio Monforte A, Cozzi-Lepri A, Rezza G *et al.* Insights into the reasons for discontinuation of the first highly active antiretroviral therapy (HAART) regimen in a cohort of antiretroviral naïve patients. I.CO.N.A. Study Group. Italian Cohort of Antiretroviral-Naïve Patients. *AIDS* 2000; **14**: 499–507.
- Ruelle J, Jnaoui K, Lefèvre I *et al.* Comparative evaluation of the VERSANT HIV-1 RNA 1.0 kinetic PCR molecular system (kPCR) for the quantification of HIV-1 plasma viral load. *J Clin Virol* 2009; **44**: 297–301.
- Paba P, Fabeni L, Ciccozzi M *et al.* Performance evaluation of the COBAS/TaqMan HIV-1 v2.0 in HIV-1 positive patients with low viral load: a comparative study. *J Virol Methods* 2011; **173**: 399–402.
- Sire JM, Vray M, Merzouk M *et al.* Comparative RNA quantification of HIV-1 group M and non-M with the Roche Cobas AmpliPrep/Cobas TaqMan HIV-1 v2.0 and Abbott Real-Time HIV-1 PCR assays. *J Acquir Immune Defic Syndr* 2011; **56**: 239–43.
- Avettand-Fènoël V, Boufassa F, Galimand J *et al.*; ANRS SEROCO Cohort Study Group. HIV-1 DNA for the measurement of the HIV reservoir is predictive of disease progression in seroconverters whatever the mode of result expression is. *J Clin Virol* 2008; **42**: 399–404.
- 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.
- 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**: 144–8.

**33** Santoro MM, Armenia D, Alteri C *et al.* Impact of pre-therapy viral load on virological response to modern first-line HAART. *Antivir Ther* 2013; **18**: 867–76.

**34** Haubrich RH, Riddler SA, Ribaldo H *et al.* Initial viral decay to assess the relative antiretroviral potency of protease inhibitor-sparing, nonnucleoside reverse transcriptase inhibitor-sparing, and nucleoside reverse

transcriptase inhibitor-sparing regimens for first-line therapy of HIV infection. *AIDS* 2011; **25**: 2269–78.

**35** Bon I, Calza L, Musumeci G *et al.* Impact of different antiretroviral strategies on total HIV DNA level in virologically suppressed HIV-1 infected patients. *Curr HIV Res* 2017; **15**: 448–55.

**36** Gandhi RT, McMahon DK, Bosch RJ *et al.*; ACTG A5321 Team. Levels of HIV-1 persistence on antiretroviral therapy are not associated with markers of inflammation or activation. *PLoS Pathog* 2017; **13**: e1006285.