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Impact of Nucleos(t)ide Reverse Transcriptase Inhibitors on Blood Telomere Length Changes in a Prospective Cohort of Aviremic HIV–Infected Adults

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Background. Tenofovir is a potent inhibitor of human telomerase. The clinical relevance of this inhibition is unknown.

Methods. A prospective cohort of human immunodeficiency virus (HIV)–infected participants with suppressed virological replication was recruited to compare whole-blood telomere length (measured by quantitative multiplex polymerase chain reaction analysis) in participants with current exposure to tenofovir disoproxil fumarate (TDF) to that in participants never exposed to TDF.

Results. A total of 172 participants were included: 67 were in the TDF group, and 105 were in the non-TDF group (75 were receiving 2 nucleosides [of whom 69 were receiving abacavir], 25 were receiving a nucleos[t]ide reverse transcriptase inhibitor [N{t}RTI]–sparing regimen, and 5 were receiving lamivudine as the only nucleoside). After 2 years, the mean blood telomere length increased significantly in the whole cohort. The TDF group had significantly smaller gains in telomere length than the non-TDF group. In the analysis restricted to participants receiving N(t)RTIs, TDF exposure was not associated with an independent negative effect. In the non-TDF group, participants treated with 2 nucleosides also had significantly smaller gains in telomere length than those receiving N(t)RTI-sparing regimens or lamivudine as the only nucleoside.

Discussion. In HIV-infected adults with prolonged virological suppression, treatment with TDF or abacavir was associated with smaller gains in blood telomere length after 2 years of follow-up.

Keywords. HIV infection; antiretroviral therapy; tenofovir; suppressed; abacavir; telomerase; telomere length.

Compared with uninfected adults, human immunodeficiency virus (HIV)–infected individuals have shorter blood telomere length (TL) [1, 2]. After HIV seroconversion, there is a rapid and substantial decrease in blood TL [3, 4]. Multiple mechanisms can contribute to TL attrition during HIV infection: inhibition of telomerase by HIV proteins such as HIV-Tat [5], chronic antigenic stimulation leading to immunosenescence (in which well-differentiated T cells with shorter telomeres predominate) [6], and direct inhibition of telomerase caused by nucleos(t)ide reverse transcriptase inhibitors (N[t]RTIs). In vitro studies have shown that the N(t)RTIs tenofovir and abacavir inhibit human telomerase, with tenofovir being the most potent inhibitor [7–9]. The clinical relevance of this in vitro finding is unknown.

To evaluate the impact of tenofovir disoproxil fumarate or other N(t)RTIs upon TL, we performed the first prospective study of blood TL changes in HIV-infected individuals in whom virological replication was suppressed during treatment with tenofovir disoproxil fumarate–containing or tenofovir disoproxil fumarate–sparing antiretroviral therapy (ART). Our research hypothesis, in line with results of in vitro studies, was that continuous exposure to tenofovir disoproxil fumarate would have a negative impact on blood TL changes.

PARTICIPANTS, MATERIALS, AND METHODS

Study Design and Population

A total of 67 HIV-infected participants currently receiving tenofovir disoproxil fumarate and 105 HIV-infected participants who had never received tenofovir disoproxil fumarate were included in this cohort. All participants were recruited from Hospital Universitario La Paz (Madrid, Spain) HIV unit between March 2014 and March 2015. Main inclusion criteria were as follows: age of >18 years, HIV antibody positive, stable ART regimen (no change in ART for at least 12 months), and serum HIV RNA load of <50 RNA copies/mL for at least 1 year prior to recruitment (a unique viral load of >50 but <200 RNA copies/mL was allowed up to 3 months before study entry). We

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offered participation in the study to all patients who met inclusion criteria in our database.

Exclusion criteria were as follows: current or previous treatment with chemotherapy or biologic agents, acute infection with systemic repercussion within the 3 weeks prior to study entry, former or active alcoholism (consumption of >70 g of alcohol per day for men and 40 g/day for women), pregnancy, and HIV infection. We obtained relevant demographic, clinical, and behavioral data and parental age at birth of the participant.

Follow-up and Variables

The study included 1 visit at study entry and 1 visit after 2 years for follow-up. Participants age, sex, race, chronic hepatitis C status (defined as a detectable hepatitis C virus RNA at study entry), and HIV-related variables (nadir $CD4^+$ T-cell count, transmission route, and AIDS stage), ART history, use of N(t)RTIs (at study entry and the follow-up visit), and use of tenofovir disoproxil fumarate at follow-up were collected from clinical records. Researchers interviewed participants about parental age at birth, financial income, education level, and use of tobacco, alcohol, and other recreational drugs (active vs former/never). No other specific intervention was performed, and participants entering the study were managed according to routine clinical practice. An income of €12 000/year (approximately $14 000/year) represented the mean income in the Spanish region where our hospital is located [10].

Laboratory Results

A blood specimen obtained after ≥8 hours of fasting was collected from all subjects at study entry and the follow-up visit at 96 weeks for real-time measurements of glucose level, creatinine level, lipid profile (including total, low-density lipoprotein, and high-density lipoprotein cholesterol levels and triglycerides level), and other biomarkers (high-sensitivity C-reactive protein [CRP] level, fibrinogen level, and D-dimer level). $CD4^+$ T-cell count and serum HIV RNA load were concomitantly measured as markers of HIV disease severity.

Ethics Statement

The study was approved by the Ethics Committee of Hospital Universitario La Paz (Madrid, Spain). Written informed consent was obtained from all participants.

TL Determination

Genomic DNA was isolated from whole-blood specimens, using the MagPurix Blood DNA Extraction Kit 1200 (Zinexts Life Science, New Tapei City, Taiwan) according to the manufacturer's instructions. Relative TL, expressed as the ratio of the telomere amplification product (T) to that of a single-copy gene (S), was determined by monochrome quantitative multiplex polymerase chain reaction (PCR) assay as described in our previous study [11]. A standard curve with genomic DNA from a pool of 3 HIV-infected participants was prepared by serial dilutions and included in each run, together with a reference sample and negative control. Study entry and follow-up samples were assayed together on the same PCR plate. All samples were run in triplicate, and those with a coefficient of variation of >15% were reanalyzed. TL for a given participant was calculated as the mean of their repeated TL measurements. The intraassay coefficients of variation for the T/S ratio, T–cycle threshold (Ct), and S-Ct were 5.74%, 0.45%, and 0.33%, respectively, whereas the interassay coefficients of variation were 7.81%, 0.78%, and 1.35%, respectively.

Statistical Analysis

Sample characteristics were described using absolute and relative frequencies for categorical and means ± standard deviations for continuous variables. Differences by treatment group were analyzed using χ^2 or Student *t* tests as appropriate.

Intention-to-continue-treatment (ITCT) analysis, ignoring treatment changes, was performed for all treatment groups at study entry. An additional as-treated (AT) analysis was done for the subgroup of participants who received their original ART regimen throughout the study. Mean annual change in TL between study entry and follow-up was calculated and modeled by means of a linear regression analysis that adjusted for the study entry TL. Because follow-up times were not equal between patients, mean annual change in TL between baseline and follow-up was calculated and further modeled by means of a linear regression analysis that adjusted for the baseline TL.

In the main analysis, we compared participants receiving tenofovir disoproxil fumarate to those not receiving tenofovir disoproxil fumarate, to analyze the effect of tenofovir disoproxil fumarate on TL change. We did this for all participants and for the subgroup of participants who received N(t)RTIs, to try to differentiate the effect of tenofovir disoproxil fumarate as compared to other N(t)RTIs. We also compared participants receiving tenofovir disoproxil fumarate to participants receiving N(t)RTI-sparing regimens. Since 3 in vitro studies have shown that lamivudine or emtricitabine do not inhibit telomerase at therapeutic concentrations [7–9], ART regimens including lamivudine as the only N(t)RTI were classified as N(t)RTI sparing. Multivariable models were used to control for confounding due to measured independent factors. We estimated the most unbiased association between tenofovir disoproxil fumarate and TL change by using an estimative approach for model selection and thus retained in the model all variables that produced a change of ≥15% in the regression coefficient for the association between tenofovir and TL. Similarly, a secondary analysis was performed to compare participants receiving any N(t)RTIs or N(t)RTIs other than tenofovir disoproxil fumarate to participants not receiving N(t)RTIs. We did not adjust *P* values for multiple comparisons.

To complement our analysis, we created a predictive model including all variables to identify which variables could be

independent predictors of TL change during follow-up. We developed a saturated model by selecting variables showing a univariate association with a P value of < .20 and sequentially dropping variables until all showed a P value of < .05. Discarded variables were reevaluated and included again if they showed an association with a P value of < .10, so we could observe effects within the limit of statistical significance.

Model assumptions were verified by means of residuals diagnostics (data not shown). The Wald test was used to derive P values.

RESULTS

Characteristics of Study Participants

Characteristics at study entry of the participants with and those without tenofovir disoproxil fumarate exposure are listed in Table 1 and Supplementary Table 3. Participants were on average predominantly male, and there were no other statistically significant differences in sex, race, income, or education level. Tenofovir disoproxil fumarate–exposed participants reported significantly greater alcohol consumption at study entry. We did not find differences in smoking or history of recreational drug use.

HIV had been acquired mainly by sexual transmission in both groups. There were no statistically significant differences in time since HIV infection diagnosis (>16 years in both groups), duration of virological suppression before enrollment, or $CD4^+$ T-cell count at study entry.

At study entry, 71.4% of participants never exposed to tenofovir disoproxil fumarate were receiving triple-drug therapy based mainly on abacavir, and 28.5% were receiving an N(t)RTI-sparing regimen. For 5 participants, N(t)RTI-sparing regimens included lamivudine as the only nucleoside.

Blood TL at Study Entry and After 2 Years of Follow-up, by Regimen

Mean TL increased 0.042 (95% CI, .004–.079) in the whole cohort (P = .030). In the ITCT analysis, blood TL at study entry was not significantly different between participants receiving and those not receiving tenofovir disoproxil fumarate. Mean annual change in blood TL was also similar in both groups (0.025 in the non–tenofovir disoproxil fumarate group and 0.014 in the tenofovir disoproxil fumarate group). However, after 2 years, the intergroup difference in mean TL was statistically significant, with a longer TL in the non–tenofovir disoproxil fumarate group (Figure 1 and Supplementary Table 1*A*). The proportion of participants who had a TL increase at follow-up was also similar in both groups. These results did not change in the AT analysis (Supplementary Table 1*B*).

At study entry, we also found that participants receiving tenofovir disoproxil fumarate or any N(t)RTIs had significantly shorter TL than those in N(t)RTI-sparing regimens. At follow-up, participants receiving N(t)RTI-sparing regimens had significantly longer TL than participants receiving tenofovir disoproxil fumarate, N(t)RTIs, or NRTI-containing regimens (Figure 1 and Supplementary Table 1*A*).

Estimative Analysis of the Impact of Different ART Regimens on Blood TL Changes

Tenofovir Disoproxil Fumarate Regimens Versus Tenofovir Disoproxil Fumarate–Sparing Regimens

In the crude ITCT analysis, exposure to tenofovir disoproxil fumarate was associated with a trend toward a negative impact on TL. After 2 years, tenofovir disoproxil fumarate–exposed participants had gains in mean blood TL that were −0.0319 inferior (95% CI, −.0648–.010) to those for participants without non–tenofovir disoproxil fumarate exposure. After adjustment for alcohol consumption, the difference in favor of the non–tenofovir disoproxil fumarate group became statistically significant, with a mean difference in TL gain of −0.0391 (95% CI, −.0729 to −.0053).

In the AT population, we found a similar estimate of the negative effect of tenofovir disoproxil fumarate exposure on TL changes. In the crude analysis, exposure to tenofovir disoproxil fumarate was associated with a nonsignificant negative trend. Again, after adjustment for alcohol consumption, the difference increased and reached statistical significance (Figure 2 and Supplementary Table 2). When we restricted the analysis to participants receiving N(t)RTIs, we did not find a significant independent effect of tenofovir disoproxil fumarate on TL changes, regardless of the analysis.

Tenofovir Disoproxil Fumarate Regimens Versus N(t)RTI-Sparing Regimens

The difference in gains in TL in favor of participants receiving N(t)RTI-sparing regimens was −0.0722 (95% CI, −.1237 to −.0207). No confounders were identified. The AT analysis showed similar results, with a difference of −0.0707 (95% CI, −.1270 to −.0144).

N(t)RTI Regimens Versus N(t)RTI-Sparing Regimens

By ITCT analysis, exposure to N(t)RTIs had a negative impact on TL changes that was statistically significant. In the crude analysis, participants not exposed to N(t)RTIs at study entry had a significantly higher mean increase in blood TL as compared to participants without exposure to N(t)RTIs at study entry. In the crude analysis, the difference in favor of participants receiving N(t)RTI-sparing regimens was −0.0592 (95% CI, −.1011 to −.0172). Similar results were found in the AT population, with a statistically significant lower gain in TL in participants exposed to N(t)RTI at study entry (mean difference, −0.0593; 95% CI, −.1027 to −.0159). Further adjustment by time since HIV infection diagnosis increased the difference in TL in favor of participants receiving N(t)RTI-sparing regimens to −0.0689.

NRTI Regimens Versus N(t)RTI-Sparing Regimens

Finally, we also compared NRTI regimens and N(t)RTI-sparing regimens. By ITCT analysis, exposure to NRTIs had a negative impact on TL changes that was statistically

Table 1. Participant Characteristics, by Treatment Regimen

Characteristic	Non-TDF[a] (n = 105)	TDF[b] (n = 67)	*P*[c]	N(t)RTI[d] (n = 142)	NRTI[e] (n = 75)	N(t)RTI Sparing[f] (n = 30)
Age, y	49.7 ± 9.8	49.4 ± 7.5	.791	49.5 ± 8.8	49.7 ± 9.9	49.8 ± 9.8
Female sex	26 (24.8)	20 (29.9)	.462	38 (26.8)	18 (24.0)	8 (26.7)
Follow-up time, mo	24.8 ± 2.4	24.3 ± 1.9	.204	24.7 ± 2.3	25.1 ± 2.5	24.0 ± 1.7
Father's age at birth, y	32.5 ± 7.0	32.8 ± 5.5	.759	32.4 ± 6.6	32.0 ± 7.4	33.9 ± 5.9
Mother's age at birth, y	29.7 ± 6.2	30.2 ± 5.5	.559	29.7 ± 6.0	29.2 ± 6.3	30.9 ± 5.9
Ethnicity						
White	96 (91.4)	64 (95.5)	.304	131 (92.3)	67 (89.3)	29 (96.7)
Other	9 (8.6)	3 (4.5)		11 (7.8)	8 (10.7)	1 (3.3)
Education level						
Primary	42 (40.0)	24 (35.8)	.527	52 (35.2)	26 (34.7)	16 (53.3)
Secondary	32 (30.5)	26 (38.8)		49 (34.5)	23 (30.7)	9 (30.0)
University	31 (29.5)	17 (25.4)		43 (30.3)	26 (34.7)	5 (16.7)
Income level						
Low	49 (46.7)	27 (40.9)	.683	63 (44.4)	36 (48.0)	13 (43.3)
High	55 (52.4)	39 (59.1)		77 (54.2)	38 (50.7)	17 (56.7)
Unknown	1 (1.0)	1 (1.5)		2 (1.4)	1 (1.3)	0 (0.0)
Current tobacco use	50 (47.6)	36 (53.7)	.434	67 (47.2)	31 (41.3)	19 (63.3)
Current alcohol use	36 (34.3)	40 (59.7)	.002	62 (43.7)	22 (29.3)	14 (46.7)
HCV coinfection at study entry						
No	72 (68.6)	42 (62.7)	.712	98 (69.0)	56 (74.7)	16 (53.3)
Active	11 (10.0)	9 (13.4)		17 (12.0)	8 (10.7)	3 (10.0)
Comorbidity						
Chronic kidney disease	5 (4.8)	1 (1.5)	.254	5 (3.5)	4 (5.3)	1 (3.3)
Hypertension	23 (21.9)	12 (17.9)	.526	29 (20.4)	17 (22.7)	6 (20.0)
Diabetes mellitus	15 (14.3)	9 (13.4)	.875	20 (14.1)	11 (14.7)	4 (13.3)
Statin receipt	37 (35.2)	15 (22.4)	.074	41 (28.9)	26 (34.7)	11 (36.7)
Route of HIV transmission						
Sexual	70 (66.7)	45 (67.2)	.670	98 (69.0)	53 (70.7)	17 (56.7)
Parenteral	31 (29.5)	21 (31.3)		41(28.9)	20 (26.7)	11 (36.7)
Unknown	4 (3.8)	1 (1.5)		3 (2.1)	2 (2.7)	2 (6.7)
Previous AIDS	59 (56.2)	37 (55.2)	.901	77 (54.2)	40 (53.3)	19 (63.3)
Time since HIV infection diagnosis, y	16.3 ± 6.4	18.1 ± 6.0	.067	16.6 ± 6.4	15.2 ± 6.5	19.1 ± 5.4
Time with HIV load <50 copies/mL, y	6.7 ± 2.8	7.2 ± 1.9	.209	6.7 ± 2.3	6.3 ± 2.6	7.4 ± 3.3
N(t)RTI regimen at study entry						
Overall	75 (71.4)	67 (100)		142 (100)	75 (100)	5 (17.0)
ABC/3TC	69 (92.0)	…		69 (48.5)	69 (92.0)	…
TDF/FTC	…	64 (95.5)		64 (45.1)	…	…
AZT/3TC	3 (4.0)	…		3 (2.1)	3 (4.0)	…
TDF + 3TC	…	2 (2.99)		2 (1.4)	…	
3TC	…	…		…	…	5 (17.0)
ABC/3TC/AZT	1 (1.33)	…		1 (0.7)	1 (1.3)	…
ABC	1 (1.33)	…		1 (0.7)	1 (1.3)	…
3TC + ddI	1 (1.33)	…		1 (0.7)	1 (1.3)	…
3TC + d4T + TDF	…	1 (1.49)		1 (0.7)	…	…
N(t)RTI-sparing regimen						
Overall	30 (28.5)	…		…	…	30 (100)
PI monotherapy	22 (73.3)	…		…	…	22 (73.3)
PI + 3TC	3 (10.0)	…		…	…	3 (10.0)
LPV/r + ETV	1 (3.3)	…		…	…	1 (3.3)
LPV/r + RAL	1 (3.3)	…		…	…	1 (3.3)
ETV + RAL	1 (3.3)	…		…	…	1 (3.3)
3TC + ETV + RAL	1 (3.3)	…		…	…	1 (3.3)
3TC + ATV + RAL	1 (3.3)	…		…	…	1 (3.3)

Table 1. Continued

Characteristic	Non-TDF[a] (n = 105)	TDF[b] (n = 67)	*P*[c]	N(t)RTI[d] (n = 142)	NRTI[e] (n = 75)	N(t)RTI Sparing[f] (n = 30)
CD4+ T-cell count, cells/mL						
At study entry	846 ± 365	744 ± 340	.069	806 ± 349	862 ± 350	806 ± 401
At follow-up	824 ± 353	772 ± 313	.331	816 ± 341	860 ± 363	741 ± 319
Change	−13 ± 223	29 ± 164	.192	14 ± 196	0.42 ± 223	−44 ± 225
HIV RNA load <50 copies/mL at follow-up	104 (100)	67 (100)	…	142 (100)	75 (100)	29 (100)

Data are no. (%) of participants or mean (± standard deviation).

Abbreviations: ABC, abacavir; ATV, atazanavir; AZT, zidovudine; d4T, stavudine; ddI, didanosine; ETV, etravirine; FTC, emtricitabine; HCV, hepatitis C virus; HIV, human immunodeficiency virus; LPV/r, lopinavir/ritonavir; N(t)RTI, nucleos(t)ide reverse transcriptase inhibitor; PI, protease inhibitor; RAL, raltegravir; TDF, tenofovir disoproxil fumarate; 3TC, lamivudine.

[a]Regimens not containing TDF.

[b]Regimens containing TDF.

[c]For comparison between the non-TDF and TDF groups.

[d]Regimens containing any N(t)RTI (except 3TC alone) or TDF.

[e]Regimens containing any nucleoside reverse transcriptase inhibitor (NRTI; ie, ABC, AZT, ddI, 3TC, or FTC) except 3TC alone.

[f]Regimens without N(t)RTIs or with 3TC only.

significant. In the crude analysis, participants not exposed to N(t)RTIs at study entry had a significantly higher mean increase in blood TL than participants with exposure to NRTIs at study entry. In the crude analysis, the difference in favor of participants receiving N(t)RTI-sparing regimens was −0.0513 (95% CI, −.0991–.0034). Similar results were found in the AT population. Further adjustment by time since HIV infection diagnosis increased the difference in TL in favor of participants receiving N(t)RTI-sparing regimens to −0.0682.

Predictive Model of Factors Associated to Annual Change in Blood TL

In the univariate analysis, older age was significantly associated with a lower mean TL gain among all participants. Female sex was associated with a trend toward a positive impact of borderline significance (Table 2 and Supplementary Figure 1). No associations with race, parental age at birth, education level, or income was observed. Regarding HIV-related factors, there was a trend toward an association between longer duration since HIV infection diagnosis and smaller increases in mean TL ($P = .051$). Exposure to N(t)RTIs at study entry was significantly

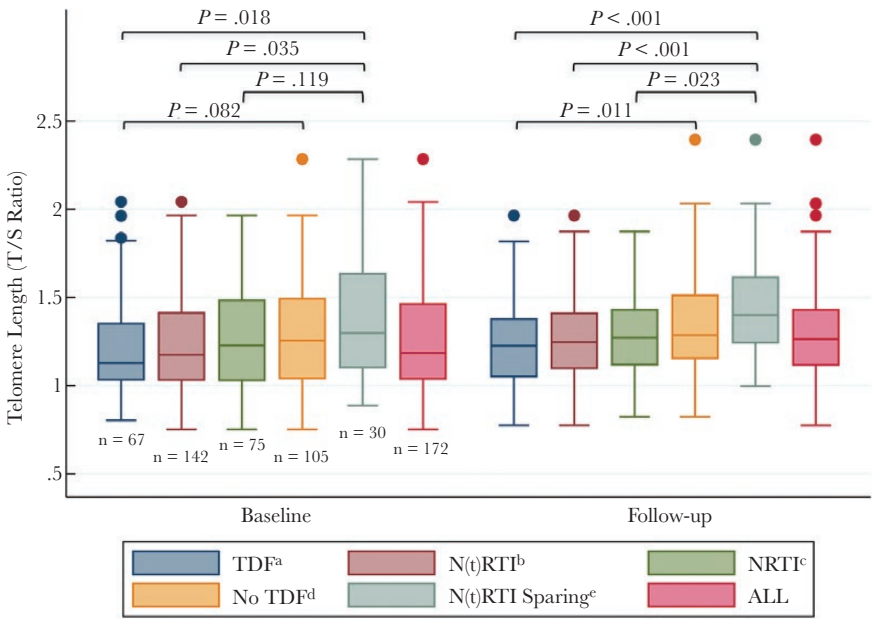

Figure 1. Intention to continue treatment analysis of blood telomere length at study entry and after 2 years of follow-up, by regimen. *P* values were determined by the Kruskal-Wallis test. N(t)RTI, nucleos(t)ide reverse transcriptase; TDF, tenofovir disoproxil fumarate; T/S ratio, ratio of the telomere amplification product to that of a single-copy gene; 3TC, lamivudine. [a]Regimens containing TDF. [b]Regimens containing any N(t)RTI (except 3TC alone) or TDF. [c]Regimens containing any nucleoside reverse transcriptase inhibitor (NRTI; ie, abacavir, atazanavir, didanosine, 3TC, or emtricitabine) except 3TC alone. [d]Regimens not containing TDF. [e]Regimens without N(t)RTIs or with 3TC only.

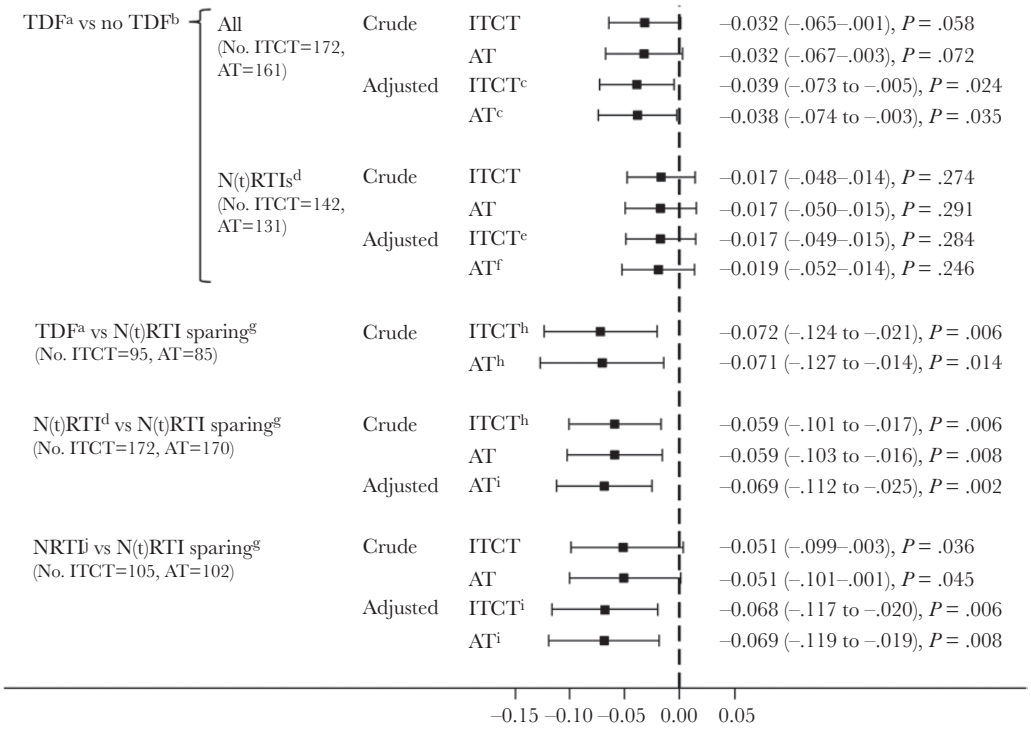

Figure 2. Mean difference in telomere length changes after 2 years, adjusted by telomere length at study entry. Telomere length was measured as mean ratio of the telomere amplification product to that of a single-copy gene. ABC, abacavir; AT, as-treated analysis (only participants without treatment changes at study entry); AZT, zidovudine; ddI, didanosine; FTC, emtricitabine; HIV, human immunodeficiency virus; ITCT, intention-to-continue-treatment analysis (ignoring treatment changes); N(t)RTI, nucleos(t)ide reverse transcriptase inhibitor; TDF, tenofovir disoproxil fumarate; 3TC, lamivudine. [a]Regimens containing TDF. [b]Regimens not containing TDF. [c]Adjusted by active alcohol consumption. [d]Regimens containing N(t)RTIs or TDF (except 3TC alone). [e]Adjusted by age, income, time since HIV infection diagnosis, active alcohol and tobacco consumption, and active hepatitis C virus infection. [f]Adjusted by income, time since HIV infection diagnosis, active alcohol and tobacco consumption, study entry fibrinogen level, change in fibrinogen level during follow-up, and statin receipt. [g]Regimens without N(t)RTIs or with 3TC only. [h]No confounding variables were identified. Therefore, only the crude estimate is shown. [i]Adjusted by time since HIV infection diagnosis. [j]Regimens containing any nucleoside reverse transcriptase inhibitors (NRTIs; ie, ABC, AZT, ddI, 3TC, or FTC) except 3TC alone. A total of 69 participants received ABC/3TC, 3 received AZT/3TC, 1 received ABC/3TC/AZT, 1 received ABC, and 1 received ddI + 3TC.

associated with a smaller annual gain in TL. Exposure to tenofovir disoproxil fumarate at study entry was associated with a trend toward lower TL gains approaching statistical significance (P = .058). An unknown mechanism of HIV transmission was significantly associated with larger annual increases in blood TL.

In the multivariable analysis, women had significantly larger annual increases in blood TL, whereas time since HIV infection diagnosis and study entry exposure to N(t)RTI had a negative influence (Table 2).

DISCUSSION

We found that, after 2 years, participants treated with tenofovir disoproxil fumarate or abacavir had significantly lower blood TL gains than participants not receiving N(t)RTIs. This is the first prospective study showing that ART including some N(t)RTIs has a measurable negative effect on longitudinal blood TL changes of HIV-infected participants with virological suppression. Since shortened TL is one of the hallmarks of senescent T cells [12], these results suggest that ART regimens including tenofovir disoproxil fumarate or abacavir could play a role in delaying recovery from HIV-associated immunosenescence.

Our in vivo results reflect in vitro findings that tenofovir disoproxil fumarate and abacavir inhibit telomerase [7–9]. The largest difference in TL gain was between participants receiving the most potent inhibitor, tenofovir disoproxil fumarate, and participants receiving N(t)RTI-sparing regimens. When we compared regimens containing abacavir—a weaker inhibitor of telomerase—to N(t)RTI-sparing regimens, the point estimate for the difference in TL gain was smaller. When comparing tenofovir disoproxil fumarate–containing regimens to abacavir-containing regimens, the point estimate of the difference was even smaller and did not reach statistical significance. Taken together, these results point toward inhibition of telomerase as the underlying mechanism responsible for the TL differences observed in this cohort.

In a previous cross-sectional study [11], we did not find an association between history of tenofovir disoproxil fumarate exposure and blood TL. Importantly, in that study 43% of participants with a history of tenofovir disoproxil fumarate use had switched to boosted protease inhibitor monotherapy at the time of the cross-sectional analysis. In contrast, all tenofovir disoproxil fumarate–exposed participants in the present prospective

Table 2. Findings of Univariate and Multivariate Analyses of the Association Between Independent Factors and Annual Change in Telomere Length

	Univariate		Multivariate	
Factor	β (95% CI)	*P*	β (95% CI)	*P*
Age, per 10 y	−0.019 (−.037 to −.0003)	.046	…	
Father's age at birth, per 10 y	0.020 (−.004–.045)	.108	…	
Mother's age at birth, per 10 y	0.005 (−.022–.032)	.720	…	
Female sex	0.036 (−.001–.072)	.054	0.042 (.007–.077)	.019
Ethnicity other than white	0.014 (−.050–.078)	.667	..	
Education level				
Primary	Reference		…	
Secondary	0.018 (−.020–.056)	.346	…	
University	0.017 (−.023–.057)	.405	…	
Income level				
Low	Reference		…	
High	0.015 (−.018–.047)	.381	…	
Route of HIV transmission				
Sexual	Reference		…	
Parenteral	0.006 (−.029–.040)	.749	…	
Unknown	0.101 (.006–.197)	.037	…	
AIDS	0.002 (−.031–.034)	.916	…	
Time since HIV infection diagnosis, per 5 y	−0.013 (−.025–.0001)	.051	−0.018 (−.030 to −.005)	.006
N(t)RTI regimen at study entry[a]	−0.059 (−.101 to −.017)	.006	−0.070 (−.111 to −.029)	.001
TDF regimen at study entry[b]	−0.032 (−.065–.001)	.058	…	
Chronic kidney disease	0.021 (−.067–.108)	.642	…	
Hypertension	−0.007(−.047–.033)	.734	…	
Diabetes mellitus	−0.013 (−.059–.034)	.594	…	
Statin receipt	0.018 (−.017–.053)	.310	…	
HCV coinfection at study entry				
No	Reference		…	
Active	−0.030 (−.081–.020)	.239	…	
Past	0.009 (−.030–.048)	.650	…	
HCV infection cured during follow-up	−0.016 (−.075–.043)	.585	…	
Current tobacco use	0.011 (−.021–.043)	.486	…	
Current alcohol use	0.019 (−.014–.0508)	.257	…	
$CD4^+$ T-cell count, per 100 cells				
At study entry	−0.0002 (−.005–.004)	.939	…	
Change	−0.0001 (−.005–.005)	.957	…	
Glucose level, per 1 SD				
At study entry	−0.007 (−.026–.012)	.454	…	
Change	−0.003 (−.020–.015)	.774	…	
Creatinine level				
At study entry	−0.008 (−.022–.006)	.270	…	
Change	0.008 (−.007–.023)	.299	…	
Total cholesterol level, per 1 SD				
At study entry	0.014 (−.002–.029)	.081	…	
Change	−0.008 (−.024–.007)	.301	…	
HDL level, per 1 SD				
At study entry	0.010 (−.006–.027)	.204	…	
Change	0.006 (−.021–.009)	.428	…	
LDL level, per 1 SD				
At study entry	0.009 (−.006–.025)	.229	…	
Change	−0.001 (−.017–.015)	.928	…	
Triglycerides level, per 1 SD				
At study entry	−0.002 (−.017–.013)	.772	…	
Change	−0.004 (−.020–.011)	.569	…	
CRP level, per 1 SD				
At study entry	0.012 (−.006–.023)	.193	…	
Change	−0.010 (−.025–.005)	.178	…	

Table 2. Continued

	Univariate		Multivariate	
Factor	β (95% CI)	*P*	β (95% CI)	*P*
Fibrinogen level, per 1 SD				
At study entry	0.004 (−.012–.020)	.606	…	
Change	−0.016 (−.032–.0001)	.052	…	
D-dimer level, per 1 SD				
At study entry	0.007 (−.007–.022)	.322	…	
Change	−0.009 (−.024–.007)	.285	…	

Analyses were adjusted by telomere length at study entry. Variables with a *P* value of < .20 were entered in the saturated model for the multivariate analysis.

Abbreviations: CI, confidence interval; CRP, C-reactive protein; HCV, hepatitis C virus; HDL, high-density lipoprotein cholesterol; HIV, human immunodeficiency virus; LDL, low-density lipoprotein cholesterol; TDF, tenofovir disoproxil fumarate.

[a]Regimens containing TDF.

[b]Regimens containing any nucleos(t)ide reverse transcriptase inhibitor (N[t]RTI; except lamivudine alone) or TDF.

cohort were receiving tenofovir disoproxil fumarate at study entry. These results suggest that the negative impact of tenofovir disoproxil fumarate on blood TL changes might be reversible after stopping tenofovir disoproxil fumarate treatment.

Our results also contradict those from a substudy of the MONET clinical trial that compared darunavir/ritonavir monotherapy to darunavir/ritonavir and 2 N(t)RTIs for maintenance of virological suppression [13] and found that continuation of N(t)RTI treatment was not associated with TL changes. However, the MONET substudy was underpowered to find an impact of N(t)RTIs on TL changes: the sample size was 124 participants, from whom samples were obtained after week 48 from only 88%, there was an imbalance at study entry, with longer TL in participants assigned to stop N(t)RTI therapy. In addition, statistical analysis did not adjust for study entry TL, as recommended for longitudinal TL studies [14].

We found that, overall, mean blood TL increased after 2 years regardless of ART strategy. This is contrary to studies performed in the general population, in which mean blood TL measured by PCR analysis showed annual decreases in blood TL [15]. This difference could imply that, despite the high $CD4^+$ T-cell counts and long duration of viral suppression, our participants are still experiencing immune reconstitution. We think it is plausible that our subgroup of participants with increases in blood TL might be shifting their T-lymphocyte subpopulations toward less mature T-cell phenotypes with longer TL [16, 17], thus translating into the increase in mean blood TL. We hypothesize that tenofovir disoproxil fumarate or abacavir can interfere with this process of immune reconstitution. It is also possible that participants receiving tenofovir disoproxil fumarate or abacavir have a similar distribution of T-lymphocyte subpopulations but with overall shorter TL. Interestingly, Cobos Jimenez et al [18] have shown that HIV-infected participants with virological suppression have shorter TL in PBMCs than well-matched controls, despite a similar distribution of senescent T cells.

This was not a randomized study, and as such, we cannot determine whether unmeasured variables could influence blood TL change. We performed analysis of multiple subgroups, increasing the likelihood of finding spurious associations with a *P* value of < .05. However, effects in all groups were highly significant and consistent with our a priori hypothesis based on in vitro data, leading us to believe that the observed associations corresponded to true effects. Another limitation is that we did not determine TL on specific subsets of T cells. Consequently, we cannot at this time prove our hypothesis that blood TL changes are driven by modifications in T-cell subpopulations. The best way to avoid some of these limitations would be to measure TL changes in different T-cell subpopulations in randomized clinical trials aiming to compare ART including 2 N(t)RTIs to N(t)RTI-sparing regimens [19, 20].

The clinical relevance of the differences in blood TL found in this cohort is unknown. One recent study in 51 injection drug users showed that, 3 months after HIV seroconversion, the TL in PBMCs measured by quantitative PCR analysis decreased 13% [3]. In our study, the mean adjusted per-protocol difference in TL gain between participants receiving and those not receiving tenofovir disoproxil fumarate at week 96 was 0.0384, representing a 3% decrease from the blood TL at study entry in the group not exposed to tenofovir disoproxil fumarate. We consider that this difference between groups is not negligible. Additionally, the differences among regimens increased at follow-up, suggesting a cumulative impact of N(t)RTIs on TL. Currently, TL is not a prognostic biomarker in persons living with HIV. However, as repeatedly reported [21, 22], TL correlates with immunosenescence in HIV-infected individuals, so the prognostic importance of blood TL in persons living with HIV could become an interesting subject of research.

The results found in this cohort of HIV-infected participants with >8 years of virological suppression are in contrast with our results from the NEAT 001 clinical trial substudy [23]. In NEAT 001, ART-naive participants treated with tenofovir disoproxil fumarate/emtricitabine and ritonavir-boosted darunavir had significant higher gains in blood TL after 2 years, compared with participants receiving a N(t)RTI-sparing regimen of raltegravir

and ritonavir-boosted darunavir. Why did tenofovir disoproxil fumarate have a negative impact on blood TL changes in our prospective cohort of participants with virological suppression but a positive impact 2 years after starting ART among participants in NEAT 001? Our hypothesis is that, in NEAT 001, the main driver of changes in blood TL was initial control of HIV replication, while in the present prospective cohort of aviremic participants, the main drivers of TL changes are both control of HIV replication and telomerase inhibition caused by tenofovir or abacavir.

Compared with tenofovir disoproxil fumarate and emtricitabine, both darunavir and raltegravir have lower concentrations in lymph node tissue [24–26]. We think that, because of these low tissue levels, participants treated with darunavir and raltegravir had a slower decay of HIV replication in lymph nodes and a persistent stimulus for T cells to differentiate into mature phenotypes with shorter TL. In contrast to NEAT 001, participants in our prospective cohort have sustained control of HIV replication. We believe it is in the scenario of prolonged suppression of viremia that inhibition of telomerase caused by tenofovir or abacavir could be most apparent and result in lower blood TL gains, as it is observed in our cohort.

In summary, in this cohort of HIV-infected participants with long-standing virological suppression, ART, regardless of the regimen, had an overall positive impact on sequential TL changes. However, N(t)RTI-sparing ART was associated with larger gains in blood TL than ART regimens containing tenofovir disoproxil fumarate or abacavir. Further research is needed to confirm whether long-term treatment with these N(t)RTIs disturbs recovery from HIV-related immunosenescence.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Potential conflicts of interest. R. M. has received payment for development of educational lectures from Janssen. N. S.-A. has received personal fees from Janssen-Cilag and from Gilead outside the submitted work. J. I. B. has received payment for lectures, including service on the speaker's bureaus, from ViiV Healthcare and Janssen; and currently receives payment for expert testimony, from Gilead Sciences and Merck Sharp and Dohme. I. P.-V. has received payment for consultancies, from Janssen-Cilag, Gilead, and ViiV Healthcare, and for lectures, including service on speaker's bureaus, from Merck Sharp & Dohme. M. L.-M. is receiving payment for board membership, from Janssen-Cilag and Abbvie, and for development of educational presentations, from Janssen-Cilag, Abbvie, and Gilead; and has received payment for consultancies, from Janssen Cilag, Abbvie, and ViiV Healthcare; for expert testimony, from Abbvie and Janssen-Cilag; and for lectures, including service on speaker's bureaus, from Janssen-Cilag, ViiV Healthcare, and Abbvie. J. R. A. is currently receiving payment for board membership and consultancies, from ViiV Healthcare, Janssen-Cilag, Gilead and Merck Sharp & Dohme; and is receiving payment for lectures, including service on speaker's bureaus, from ViiV Healthcare, Janssen Cilag, Gilead, and Merck Sharp & Dohme. All other authors report no potential conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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