MAJOR ARTICLE



Blood Telomere Length Changes After Ritonavir-Boosted Darunavir Combined With Raltegravir or Tenofovir-Emtricitabine in Antiretroviral-Naive Adults Infected With HIV-1

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

Methods. NEAT001/ANRS143 is a randomized trial that showed noninferiority over 96 weeks of ritonavir-boosted darunavir plus raltegravir versus tenofovir disoproxil fumarate/emtricitabine in 805 antiretroviral antiretrovrial-naive HIV-infected adults. We compared changes in whole-blood telomere length measured with quantitative polymerase chain reaction in 201 randomly selected participants (104 raltegravir and 97 tenofovir disoproxil fumarate/emtricitabine). We performed multivariable estimative and predictive linear regression.

Results. At week 96, participants receiving tenofovir disoproxil fumarate/emtricitabine had a statistically significant higher gain in telomere length than participants receiving raltegravir. Difference in mean telomere length change between groups (tenofovir disoproxil fumarate/emtricitabine minus raltegravir) from baseline to week 96 adjusted by baseline telomere length was 0.031 (P = .009). This difference was not significantly confounded by age, gender, known duration of HIV infection, CD4 (baseline/nadir), CD8 cells, CD4/CD8 ratio, HIV viral load (baseline/week 96), tobacco and alcohol consumption, statins, or hepatitis C.

Conclusion. Antiretroviral-naive HIV-infected adults receiving ritonavir-boosted darunavir and tenofovir disoproxil fumarate/ emtricitabine had a significant higher gain in blood telomere length than those receiving ritonavir-boosted darunavir and raltegravir, suggesting a better initial recovery from HIV-associated immunosenescence.

Keywords. HIV infection; antiretroviral therapy; darunavir/ritonavir; raltegravir; tenofovir; telomere length; telomerase.

Human immunodeficiency virus (HIV) infection leads to an accelerated immunosenescence status marked by dominant senescent and exhausted phenotypes of mature T cells with a decrease in naive T cells [1, 2]. Senescent T cells have limited proliferative capacity due to telomere attrition [3]. In keeping with this immunosenescence status, HIV-infected individuals have shorter blood telomere length (TL) than HIV-uninfected controls [4–7].

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Antiretroviral treatment (ART) partially reverses HIVassociated immunosenescence. Initial control of HIV replication translates into an increase in naive and central memory CD4 and CD8 cells that have longer telomeres. The increase in TL after initiating ART is correlated mainly with shifts of CD8 cells subpopulations towards less mature phenotypes [8, 9].

In vitro studies have shown that tenofovir and abacavir, 2 recommended nucleos(t)ide reverse transcriptase inhibitors (N(t) RTI), are able to inhibit human telomerase, tenofovir being the most potent inhibitor [10–12]. The clinical relevance of this in vitro finding is unknown. There are no studies comparing TL changes in ART-naive participants who start treatment with N(t)RTI-containing versus N(t)RTI-sparing ART. For this reason, we have evaluated blood TL changes in a substudy of the NEAT001/ANRS 143 clinical trial that compared ritonavir-boosted darunavir combined with raltegravir or tenofovir disoproxil fumarate/emtricitabine in ART-naive adults. Our research hypothesis was that exposure to tenofovir, in line with its in vitro activity inhibiting the telomerase, would have a negative impact on blood TL changes.

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METHODS

Study Design and Participants

NEAT001/ANRS143 was a randomized 1:1, open-label, 96 week, noninferiority trial conducted in 78 clinical sites in 15 European countries between August 2010 and October 2013. Ethics committee and competent authority approval was obtained for all participating centers, in accordance with the principles of the Declaration of Helsinki. Inclusion criteria were: HIV RNA greater than 1000 copies per mL and CD4 cell count under 500 cells per μ L in ART-naive participants (the full study design and patient population have been previously described) [13]. Participants were excluded if they presented any of the following: treatment for malignant disease, positive hepatitis B virus surface antigen, pregnancy, creatinine clearance of less than 60 mL/min or any other relevant laboratory abnormalities.

Randomization and Masking

Randomization of the parent study was performed as previously reported [13]. We randomly assigned participants (1:1) to receive oral treatment with either 800 mg darunavir and 100 mg ritonavir once per day plus 400 mg raltegravir twice daily (N(t)RTI-sparing regimen) or 800 mg darunavir and 100 mg ritonavir plus tenofovir disoproxil fumarate 245 mg and 200 mg emtricitabine in a fixed-dose combination once per day (standard regimen). Tenofovir disoproxil fumarate/emtricitabine was provided by Gilead Sciences, darunavir by Janssen Pharmaceuticals, and raltegravir by Merck Laboratories. Participants included in this substudy were randomly selected stratified by treatment group using Stata software (version 14.0; Stata Corporation, College Station, TX).

Participants were applicable for enrolment in this substudy if they had consented to biobank preservation and had available blood samples from both baseline and week 96. Of the total of 805 participants enrolled in NEAT 001, 681 participants had appropriate blood samples at both baseline and 96 weeks. Because there were no prior data on which to base sample size calculation, of these 681 participants we selected an initial random sample of 100 subjects for TL determinations. This initial blinded comparison revealed significant differences between groups (Supplementary Table 1). In order to have a more precise estimate of the differences and to confirm our findings, the sample size was further increased afterwards with an additional randomly selected 100 subjects. We obtained written informed consent from all participants for the parent study.

Telomere Length Determination by Quantitative Real-Time PCR

Genomic DNA was isolated from whole blood using QIAamp DNA Blood Mini Kit (Qiagen) according to manufacturer's instructions. Relative telomere length, expressed as ratio of telomere (T) to single-copy gene (S), was determined by monochrome quantitative multiplex polymerase chain reaction (PCR) assay, with minor modifications as described in our prior study [14]. A standard curve was prepared with genomic DNA from a pool of 3 healthy volunteers by serial dilution and was included in triplicate in each run together with a reference sample and negative control. Baseline and follow-up samples were assayed in triplicate on the same PCR plate and those with a coefficient of variation (CV) greater than 10% were reanalyzed. The intra-assay CV was calculated as the average of the individual CVs of the samples and the interassay CV using the reference sample. The intra-assay coefficient of variation for T/S ratio, T-cycle threshold and S-cycle threshold were 4.7%, 0.4%, and 0.3%, and the interassay CV were 5.7%, 1.0%, and 0.8%, respectively

Outcomes

The primary outcome of the telomere substudy was the change in TL from baseline to week 96. The secondary outcomes were increase in the TL and increase greater than 1 standard deviation (SD) in TL from baseline to week 96.

Covariates included in the analysis were gender (male, female), age, ethnicity (Asian, Black, Caucasian, other), and alcohol (non/moderate, exdrinker, current drinker, unknown) and tobacco (never, stopped, currently) consumption at baseline, hepatitis C virus (HCV) coinfection (negative, nonactive, active, unknown), statin treatment (yes, no), HIV mechanism of acquisition (homo/bisexual, heterosexual, others, unknown), time since HIV diagnosis, HIV CDC clinical stage (A, B, C), CD4 cell count, nadir CD4 cell count, CD8 cell count, CD4/CD8 ratio, log_{10} HIV-1 RNA, HIV-1 RNA (<100 000 HIV-RNA copies/mL, $\geq 100 000$ HIV-1 RNA copies/mL), and baseline TL.

Statistical Analyses

Characteristics of the participants with available samples (n = 681) and those included in the substudy (n = 201) were described using absolute and relative frequencies and mean (standard deviation) for categorical and continuous variables, respectively. Balance in the treatment group was analyzed using X^2 or median tests accordingly. We calculated the mean increases and 95% confidence intervals in TL from baseline to week 96. We performed a multivariable estimative linear regression model to assess differences in TL increases by treatment group adjusting for baseline TL and potential confounders (retaining in the model variables that produced a change ≥15% in the treatment mean difference). We also performed a multivariable predictive linear regression model to identify predictive factors for TL change. All variables that retained a significant independent association (P < .01) were included in the final model. We used multivariable logistic regression models to assess differences by treatment group in the proportion of participants attaining a TL increase and a TL increase >1 SD from baseline to week 96.

As a complementary analysis, we used Kaplan-Meier method to calculate probability of reaching virological response (HIV-1

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RNA < 50 copies/mL) and CD4/CD8 ratio normalization (CD4/ CD8 > 0.4). Wald tests were used to derive *P* values. All statistical analyses were performed using Stata software (version 14.0; Stata Corporation, College Station, TX).

RESULTS

Characteristics of Study Participants

Telomere length was analyzed on 201 random participants, 97 in the tenofovir disoproxil fumarate/emtricitabine exposed group and 104 in the raltegravir exposed group. Baseline characteristics were all well balanced between groups, with no notable differences from the total telomere substudy samples (Table 1). The great majority of participants were male. There were no statistically significant differences in gender, age, or race. Both groups had acquired HIV mainly by sexual transmission and the mean time since HIV diagnosis was 2.1 years. We did not find differences in tobacco or alcohol consumption. There were no statistically significant differences in CD4 cell count, CD4 nadir, CD8 cell count, CD4/CD8 ratio, HIV-1 RNA load, HCV coinfection, or treatment with statins.

At week 96, we did not find differences between groups in immunovirologic response (Table 2). More than 90% of participants had achieved virological suppression in both treatment arms. There were not statistically significant differences in the proportion of participants reaching the primary endpoint of the core trial (virologic nonresponse or failure, death AIDS event, or serious non-AIDS event). The number of virological endpoints was the same in each arm. Time to virological response was significantly shorter for participants randomized to raltegravir (Table 2 and Supplementary Figure 1). There were no statistically significant differences in any of the immunological parameters measured at week 96: CD4 change, CD8 change, CD4/CD8 change, proportions with CD4/CD8 above 0.4 or 1, and time to achieve a CD4/CD8 ratio >0.4 (Table 2 and Supplementary Figure 2).

Blood Telomere Length Changes

Blood telomere length analysis showed that at baseline there were no statistically significant differences between groups. After 96 weeks, both groups had a gain in TL: mean TL in the total analyzed samples increased by 0.028 (Figure 1 and Supplementary Table 2). However, the increase in TL was only significant in the tenofovir disoproxil fumarate/emtricitabine group. Additionally, the proportion of participants who had increase in TL at follow-up was 71% in tenofovir disoproxil fumarate/ emtricitabine group versus 57% in the raltegravir group.

Multivariable Estimative Analysis

In our estimative analysis, exposure to tenofovir disoproxil fumarate/emtricitabine had a positive impact on TL change. After 96 weeks, tenofovir disoproxil fumarate/emtricitabine– exposed participants had a gain in mean blood TL adjusted by baseline TL that was 0.031 superior to raltegravir-exposed participants (P = .009) (Figure 2). This effect was not significantly confounded by age, gender, race, time since HIV diagnosis, baseline HIV RNA, nadir or baseline CD4 cell count, baseline CD8, baseline CD4/CD8, tobacco and alcohol consumption, statins, or hepatitis C. These results were unchanged when TL was analyzed as a binary variable (TL shortened/not shortened; data not shown).

Predictive Model

In the univariate analysis treatment with tenofovir disoproxil fumarate/emtricitabine, younger age and no current alcohol consumption were significantly associated with a gain in mean TL among all participants. We found no significant associations with tobacco, gender, race, or statin treatment. Regarding HIVrelated factors, we found no significant associations of mean TL gain with time since HIV diagnosis, nadir or baseline CD4, baseline HIV RNA, and HCV coinfection (Table 3).

In the multivariable analysis, independent predictors of gain in TL were baseline TL (P < .001), treatment with tenofovir disoproxil fumarate/emtricitabine (P = .005), and no current alcohol consumption at baseline (P = .026). There was a trend (P = .097) towards younger age also being associated with higher gains in TL (Table 3).

DISCUSSION

Surprisingly, and contrary to our research hypothesis based on in vitro study results [10–12], participants receiving ritonavir-boosted darunavir, emtricitabine, and tenofovir disoproxil fumarate had significantly higher gains in TL than those receiving a N(t)RTI-sparing regimen (ritonavir-boosted darunavir and raltegravir). This is the first clinical trial showing that N(t)RTI-containing ART has a measurable positive impact on longitudinal TL changes, in naive HIV participants. This result suggests that ART regimens including N(t)RTIs could play an important role in initial recovery from HIV-associated immunosenescence.

Although overall mean blood TL increased after 2 years regardless of ART, this was only statistically significant in participants randomized to tenofovir disoproxil fumarate/emtricitabine. The difference in mean gain in TL was also statistically significant in favor of tenofovir disoproxil fumarate/emtricitabine, and the proportion of participants with increases in TL was 14% higher than the N(t)RTI-sparing group. In our estimative analysis the adjusted difference between groups in mean TL changes was 0.031, which represents 4.2% of the baseline mean blood TL for the whole population. One recent study in 51 intravenous drug users showed that 3 months after HIV seroconversion the TL in peripheral blood mononuclear cells measured by quantitative PCR decreased 13% [15]. Therefore, it is likely that the difference between arms in recovery of blood TL after starting ART is important.

Table 1. Baseline Characteristics of Study Participants

	RAL+DRV/r (n = 104)	TDF/FTC+ DRV/r+	PValue
		(n = 97)	
Gender, n (%)			.862
Female	11 (10.6)	11 (11.3)	
Age, median years (IQR)	37.6 (30.5–46.3)	37.3 (30.7–46.4)	.722
Ethnicity, n (%)			.206
Asian	2 (1.9)	1 (1.0)	
Black	11 (10.6)	12 (12.4)	
Caucasian	90 (86.5)	78 (80.4)	
Other	1 (1.0)	6 (6.2)	
Mode HIV transmission, n (%)			.691
Homosexual/bisexual	72 (69.2)	69 (71.1)	
IVDU (since 1984)	0 (0.0)	1 (1.0)	
Heterosexual	24 (23.1)	22 (22.7)	
Other	1 (1.0)	0 (0.0)	
Unknown	7 (6.7)	5 (5.2)	
Smoking, n (%)			.417
Never	61 (58.7)	48 (49.5)	
Stopped	8 (7.7)	10 (10.3)	
Currently	35 (33.7)	39 (40.2)	
Alcohol, n (%)			.197
Non/moderate drinker	99 (95.2)	86 (88.7)	
Exdrinker	1 (1.0)	1 (1.0)	
Current drinker	4 (3.8)	10 (10.3)	
Time since HIV diagnosis, median years (IQR)	1.3 (0.4–2.7)	1.4 (0.2–2.8)	.887
HIV CDC clinical stage, n (%)			.455
A	87 (83.7)	87 (89.7)	
В	12 (11.5)	7 (7.2)	
С	5 (4.8)	3 (3.1)	
CD4 cell count, cells per µL, n (%)			.866
<50	5 (4.8)	3 (3.1)	
50–199	14 (13.5)	12 (12.4)	
200–349	36 (34.6)	40 (41.2)	
150–499	42 (40.4)	37 (38.1)	
≥500	7 (6.7)	5 (5.2)	
CD4 cell count, median cells per µL (IQR)	346 (267–425)	333 (246–400)	.609
Nadir CD4 cell count, median cells per µL (IQR)	333 (245–394)	300 (232–360)	.069
CD8 cell count, median cells per µL (IQR)	886 (656–1205)	839 (622–1085)	.276
CD4/CD8 ratio, median (IQR)	0.4 (0.2–0.5)	0.4 (0.3–0.5)	.561
HIV-1 RNA, median log10 cop/ mL), (IQR)	4.7 (4.5–5.2)	4.7 (4.5–5.1)	.834
HIV-1 RNA, n (%)			.568
<100 000 copies/mL	69 (66.3)	68 (70.1)	
≥100 000 copies/mL	35 (33.7)	29 (29.9)	
HCV coinfection, n (%)			.472
Negative	103 (99.0)	94 (96.9)	
Nonactive	1 (1.0)	2 (2.1)	
Active	0 (0.0)	1 (1.0)	
Statins, n (%)	9 (8.7)	7 (7.2)	.707

Abbreviations: DRV/r, darunavir/ ritonavir; FTC, emtricitabine; IQR, interquartile range; IVDU, intravenous drug use; RAL, raltegravir; TDF, tenofovir disoproxil fumarate.

The positive impact of tenofovir disoproxil fumarate/emtricitabine on TL gain was not confounded by baseline or week 96 variables. In our predictive analysis the only factors associated with a statistically higher gain in TL were tenofovir disoproxil fumarate/emtricitabine and no current alcohol consumption, with younger age approaching significance. Alcohol abuse has been previously associated with TL attrition in HIV-negative individuals [16].

To the best of our knowledge this is the first clinical trial that compares TL changes after initiation of 2 different ART strategies. Two small prior studies have reported that participants starting ART experienced increases in mean TL [8, 17] driven by

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Table 2. Week 96 Characteristics of Participants Included in the Substudy

	RAL+DRVV/r	TDF/FTC+DRV/r	<i>P</i> Value
Median and IQR	(n = 104)	(n = 97)	
Total participants meeting primary endpoint during follow-up, n (%)	18 (17.3)	16 (16.5)	.878
Virologic endpoint, , n (%)			
HIV RNA ≥50 copies/mL at week 32	5 (4.8)	11 (11.3)	.121
HIV RNA ≥50 copies/mL after week 32	9 (8.7)	3 (3.13)	
HIV RNA concentration <50 copies per mL, n (%)	99 (95.2)	89 (91.8)	.322
Time to HIV RNA <50 copies per mL, median weeks (IQR)	8 (4–12.6)	18 (9.4–24.1)	<.001
CD4 cell count, cells/mm ³	597.06 (202.15)	568.39 (206.38)	.323
CD4 cell count change, cells/mm ³	265.52 (159.64)	253.40 (167.43)	.602
CD8 cell count, cells /mm ³	811.36 (329.03)	811.54 (410.93)	.997
CD8 cell count change, cells /mm ³	-123.90 (442.25)	-124.89 (350.35)	.987
CD4/CD8 ratio	0.83 (0.36)	0.82 (0.40)	.333
CD4/CD8 ratio change	0.35 (0.82)	0.43 (0.29)	.855
% with CD4/CD8 >0.4, n (%)	94 (90.4)	82 (85.4)	.269
Time to CD4/CD8 >0.4, median weeks (IQR)	24 (0.1–25)	24 (0.1–25)	.551
% with CD4/CD8 >1, n (%)	35 (33.7)	29 (29.9)	.604

Abbreviations: DRV/r, darunavir/ ritonavir; FTC, emtricitabine; IQR, interquartile range; IVDU, intravenous drug use; RAL, raltegravir; TDF, tenofovir disoproxil fumarate.

a uniform increase in the TL of CD8 T cells that correlated with a decrease in mature memory cells. Changes in the TL of CD4 T cells were more inconsistent and variable. Given these results, our hypothesis for the observed differences in TL between the two strategies in our study is that participants receiving tenofovir disoproxil fumarate/emtricitabine experienced larger increases in the TL of mainly CD8 cells and that this increase represents a shift towards less-mature T8 cell phenotypes with longer TL. Support for this hypothesis comes from the fact that 6 months after starting ART there is a decrease in proportion of CD28⁻CD8⁺ that characteristically have shorter TL and an increase in central memory T cells that have longer TL [9]. Interestingly the other predictive factor of lower TL gain in our study, alcohol consumption, increases CD8⁺ T-cell



Figure 1. Blood telomere length in naive HIV-participants starting antiretroviral treatment. Abbreviations: DRV/r, darunavir/ ritonavir; FTC, emtricitabine; RAL, ral-tegravir; TDF, tenofovir disoproxil fumarate; T/S, telomere to single-copy gene ratio.

immunosenescence in simian immunodeficiency virus-infected rhesus macaques [18].

After starting ART, the main driver for immune reconstitution and a shift towards T-cell subpopulations with longer TL (naive and central memory) is the decrease of antigenic stimulation secondary to rapid control of HIV replication. In the parent NEAT 001/ANRS 143 trial although the N(t)RTI sparing regimen met noninferiority criteria for the primary endpoint, there were important differences in efficacy in favor of the tenofovir disoproxil fumarate/emtricitabine arm in the subgroup of participants with viral loads above 100000 HIV RNA copies/ mL and/or CD4 cell counts under 200 cells/µL [13]. It is therefore possible that differences in TL between groups in our substudy could be due to worse virological control with the N(t) RTI-sparing strategy. In our random sample of participants differences in blood TL occurred despite both groups having similar control of plasma viral replication and similar number of primary and virological endpoints. Notwithstanding, at week 96 plasma virological suppression was numerically higher, and occurred sooner, in participants receiving raltegravir than in those receiving tenofovir disoproxil fumarate/emtricitabine. A possible explanation is that plasma viral load may not completely reflect the antiviral efficacy of ART in tissues, especially in lymph nodes. Three recent studies have reported that compared to tenofovir disoproxil fumarate and emtricitabine, both darunavir and raltegravir have lower concentrations in lymph node tissue [19-21]. We hypothesize that participants receiving darunavir and raltegravir could have persistent HIV replication in lymph nodes (due to lower tissue concentrations) than participants receiving darunavir and tenofovir disoproxil fumarate/ emtricitabine. This persistent antigenic stimulation in lymph nodes would maintain the stimulus for T cells to differentiate to



Figure 2. Mean differences between groups in telomere length (TL) change at week 96. Unadjusted and adjusted by confounders (all adjusted by baseline TL). TL measured as ratio of telomere to single-copy gene (T/S). Mean differences (95% confidence interval [CI]) from estimative multivariable regression models with TL change at week 96 as the dependent variable, treatment group as main exposure variable and each potential confounder: gender (male, female), age, ethnicity (Asian, Black, Caucasian, other) alcohol (non/moderate, exdrinker, current drinker, unknown) and tobacco (never, stopped, currently) consumption, HCV coinfection (negative, nonactive, active, unknown), statin treatment (yes, no), HIV mechanism of acquisition (homo/bisexual, heterosexual, others, unknown), time since HIV diagnosis, HIV CDC clinical stage (A, B, C), CD4 cell count, nadir CD4 cell count, CD8 cell count, CD4/CD8 ratio, log10 HIV-1 RNA, HIV-1 RNA (<100 000 c/mL). All the models were also adjusted by baseline TL. Abbreviations: DRV/r, darunavir/ ritonavir; FTC, emtricitabine; RAL, raltegravir; TDF, tenofovir disoproxil fumarate.

	Univariate		Multivariable	
	Mean Differenceª (95% CI)	Р	Mean Difference (95% CI)	Р
TDF/FTC	0.031 (0.008–0.054)	.009	0.033 (0.010–0.056)	.005
Gender (ref. male)	0.016 (-0.022 to 0.053)	.410		
Younger age (per 10 years)	0.013 (0.025–0.000)	.042	0.001 (0.000-0.002)	.097
Ethnicity (ref. Asian)				
Black	0.048 (-0.053 to 0.149)	.351		
Caucasian	0.022 (-0.074 to 0.118)	.651		
Other	-0.022 (-0.136 to 0.092)	.707		
Tobacco (ref. never)				
Stopped	-0.024 (-0.066 to 0.018)	.263		
Currently	0.007 (-0.018 to 0.032)	.594		
Alcohol (ref. non/moderate)				
Exdrinker	0.075 (-0.041 to 0.191)	.202	0.073 (-0.040 to 0.186)	.205
Current drinker	-0.048 (-0.094 to -0.003)	.038	-0.052 (-0.097 to -0.006)	.026
Statin treatment (ref. No)	-0.007 (-0.051 to 0.037)	.755		
HIV mechanism of acquisition (homo/bisexual)				
Heterosexual	0.015 (-0.013 to 0.043)	.287		
Other	-0.003 (-0.121 to 0.115)	.965		
Time since HIV diagnosis (years)	0.001 (-0.002 to 0.005)	.481		
HIV-1 RNA ≥ 100 000 copies/mL	-0.012 (-0.038 to 0.013)	.339		
Nadir CD4 cell count (per 100 cells)	0.003 (-0.007 to 0.013)	.612		
Baseline CD4 cell count (per 100 cells)	0.002 (-0.007 to 0.011)	.637		
Baseline CD8 cell count (per 100 cells)	0.001 (-0.002 to 0.003)	.566		
Baseline CD4/CD8 ratio	0.001 (-0.020 to 0.021)	.956		
HCV coinfection (ref. negative)				
Active	-0.016 (-0.112 to 0.081)	.751		
Nonactive	0.103 (-0.063 to 0.268)	.222		
Baseline TL	-0.290 (-0.367 to -0.214)	<.001	-0.324 (-0.408 to -0.241)	<.001

Table 3. Predictive Univariate and Multivariable Models for Determinants of Telomere Length Change

Abbreviations: FTC, emtricitabine; TDF, tenofovir disoproxil fumarate; TL, telomere length. ^aAdjusted by baseline telomere length.

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mature phenotypes with shorter TL. In naive participants with high levels of HIV replication a higher penetration of tenofovir disoproxil fumarate/emtricitabine in lymph node tissue may overcome the inhibitory effect of tenofovir upon telomerase [10–12]. We have shown that, in the setting of virological suppression, tenofovir has a negative impact on longitudinal TL changes, [22] further supporting that in naive participants the main driver of immunosenescence is active HIV replication.

An alternative explanation for our findings is that differences in blood TL indicate an increase in T cells with shorter TL in participants treated with darunavir and raltegravir due to better control of virological replication and earlier decrease of immune activation. Individuals chronically infected with HIV have low proportions of CD28⁻CD8⁺ T cells expressing CD57, which are characterized by very short telomeres [23]. After ART initiation, the proportion of CD28-CD8+ T cells completing terminal differentiation and expressing CD57 increases [24]. Therefore, is possible that participants treated with darunavir and raltegravir do not experience blood TL increase due to lower immune activation and increasing numbers of CD28⁻CD57⁺CD8⁺ T cells. We consider this possibility less likely: firstly because overall results of NEAT 001 indicate lower efficacy of the darunavir and raltegravir regimen, and secondly because the net effect of successful ART is to increase TL (as has been seen in several studies [8, 17], including our prospective cohort of virologically suppressed participants) [22].

Significant differences in blood TL occurred despite similar changes in CD4 and CD8 cell counts and similar CD4/CD8 ratios. In virologically suppressed participants with CD4 cell counts above 500 cells/µL, the CD4/CD8 ratio is correlated positively with the frequency of T cells with longer telomeres (naive T cells, central memory CD8, and transitional memory CD8) and negatively with the frequency of T cells with shorter telomeres (effector memory and terminally differentiated cells) [25]. However, in our study, despite the large difference observed in TL by treatment arm, there were no differences in CD4/CD8 ratio or in time to achieve a CD4/CD8 ratio above 0.4, a cutoff that in one study identified individuals with prominent immunosenescence [25]. Our data suggest that the CD4/CD8 ratio may not be sensitive enough to identify differences in the distribution of T-cell subpopulations with different TL within the first 2 years of initial ART. In our study we unveil important TL differences between 2 different ART strategies despite similar control of viral replication in blood and similar changes in CD4, CD8, and CD4/CD8 ratios. Given these results, the use of blood TL to evaluate the immunological impact of initial ART could become an interesting subject of research.

The main limitation of our study is that we did not determine TL on specific subsets of T cells. Consequently, we cannot prove at this time our hypothesis that blood TL changes are driven by modifications in T-cell subpopulations. The other limitation is the lack of samples beyond week 96. Without these long-term samples it is not possible to evaluate the long-term evolution of the observed differences between study arms. In summary, in antiretroviral-naive participants, N(t)RTIsparing ART using ritonavir-boosted darunavir and raltegravir was associated with lower longitudinal gains in blood TL than N(t)RTI containing ART using ritonavir-boosted darunavir, emtricitabine and tenofovir disoproxil fumarate. These results suggest that N(t)RTI-containing ART produces a more rapid initial recovery from HIV-associated 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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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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