

Comparison of Changes in Bone Density and Turnover with Abacavir-Lamivudine versus Tenofovir-Emtricitabine in HIV-Infected Adults: 48-Week Results from the ASSERT Study

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(See the editorial commentary by Carr and Hoy, on pages 973–975.)

Background. Abacavir-lamivudine and tenofovir DF-emtricitabine fixed-dose combinations are commonly used as first-line antiretroviral therapies. However, few studies have comprehensively compared their relative safety profiles.

Methods. In this European, multicenter, open-label, 96-week study, antiretroviral-naïve adult subjects with human immunodeficiency virus (HIV) infection were randomized to receive either abacavir-lamivudine or tenofovir-emtricitabine with efavirenz. Primary analyses were conducted after 48 weeks of treatment. Bone mineral density (BMD), a powered secondary end point, was assessed by dual energy x-ray absorptiometry. Bone turnover markers (osteocalcin, procollagen 1 N-terminal propeptide, bone specific alkaline phosphatase, and type 1 collagen cross-linked C telopeptide [CTX]) were assessed in an exploratory analysis.

Results. A total of 385 subjects were enrolled in the study. BMD loss was observed in both treatment groups, with a significant difference in the change from baseline in both total hip (abacavir-lamivudine group, -1.9% ; tenofovir-emtricitabine group, -3.6% ; $P < .001$) and lumbar spine (abacavir-lamivudine group, -1.6% ; tenofovir-emtricitabine group, -2.4% ; $P = .036$). BMD loss of $\geq 6\%$ was more common in the tenofovir-emtricitabine group (13% of the tenofovir-emtricitabine group vs 3% of the abacavir-lamivudine group had a loss of $\geq 6\%$ in the hip; 15% vs 5% had a loss of $\geq 6\%$ in the spine). Bone turnover markers increased in both treatment groups over the first 24 weeks, stabilizing or decreasing thereafter. Increases in all markers were significantly greater in the tenofovir-emtricitabine treatment group than in the abacavir-lamivudine group at week 24. All but CTX remained significantly different at week 48 (eg, osteocalcin: abacavir-lamivudine group, $+8.07$ mg/L; tenofovir-emtricitabine group, $+11.92$ mg/L; $P < .001$).

Conclusions. This study demonstrated the impact of first-line treatment regimens on bone. Greater increases in bone turnover and decreases in BMD were observed in subjects treated with tenofovir-emtricitabine than were observed in subjects treated with abacavir-lamivudine.

Because the treatment of human immunodeficiency virus (HIV) infection is currently life long, the long-term

toxicity profile of antiretroviral therapy (ART) regimens is of major importance. Minimizing long-term toxicity and maximizing the adherence to a treatment regimen and the treatment response are critical therapy objectives.

At the time of this study, 2 fixed-dose combinations (abacavir sulfate [600 mg] in combination with lamivudine [300 mg] and tenofovir disoproxyl fumarate [300 mg] in combination with emtricitabine [200 mg])

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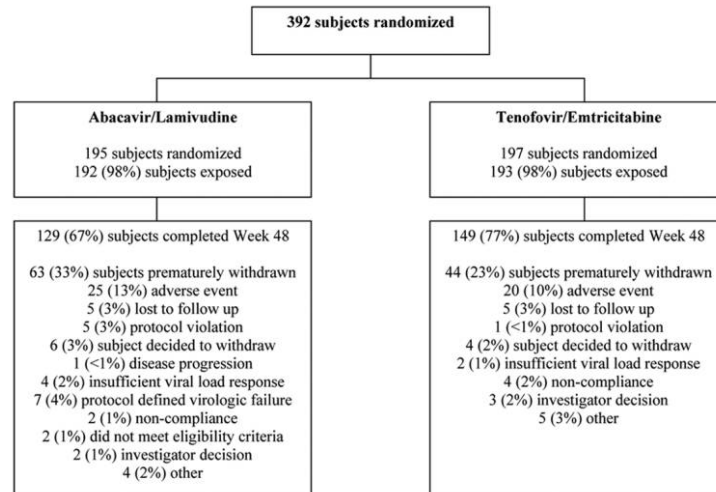


Figure 1. Summary of subject disposition.

were recommended for initial use in treating ART-naïve, HIV-infected subjects [1, 2]. Both combinations can effectively treat HIV infection, but their toxicity profiles are different. Abacavir is associated with a hypersensitivity reaction [3, 4], which can be largely avoided with *HLA-B*5701* screening [5]. Recently, an increased risk of myocardial infarction associated with abacavir use has been reported [6]; however, data from different studies have yielded inconsistent results [7, 8]. Although the incidence of nephrotoxicity in clinical studies of tenofovir has been relatively low, several studies have shown an association of tenofovir with bone abnormalities (eg, loss of bone mineral density [BMD] [9, 10], hypophosphatemic osteomalacia [11], and increases in serum alkaline phosphatase levels [12]).

Bone disease in HIV-infected patients is of increasing concern. A high proportion of patients are reported to have low BMD [13–19], and fracture prevalence rates are higher among HIV-infected patients than they are among non-HIV-infected patients [16, 20]. In studies involving treatment-naïve patients, initiation of ART has consistently been associated with a loss of BMD, with some regimens being associated with a greater loss of BMD than other regimens [13, 19, 21–23]. In addition to tenofovir, treatment with protease inhibitors has often been associated with greater BMD loss than that associated with other therapies [13, 14, 16, 19], and treatment with zidovudine-lamivudine has recently been reported to cause greater BMD loss than nevirapine [21].

Bone turnover markers are indicators of bone remodeling and reflect the rate of bone formation and resorption. Acceleration of bone turnover plays an established role in the pathogenesis of metabolic bone diseases, including osteoporosis, and is associated with an increase in markers of resorption and formation [24]. Key bone turnover markers include:

- Osteocalcin (OC): a vitamin K-dependent protein that is

synthesized and secreted by osteoblasts, and which is a specific marker of osteoblast function. Serum levels correlate well with bone formation [25].

- Bone-specific alkaline phosphatase (BSAP): an osteoblast enzyme that is thought to be involved in the mineralization of osteoid [25]. Serum concentrations demonstrate a linear relationship with osteoblast activity.
- Procollagen type 1 N-terminal propeptide (P1NP): cleaved from procollagen as it is incorporated into bone matrix, with P1NP released into the blood. Most serum P1NP originates from bone formation [25].
- Type I collagen cross-linked C-telopeptide (CTX): a marker of collagen degradation, and a biochemical indicator of bone resorption [26].

This study was designed to evaluate the renal and bone safety of abacavir-lamivudine and tenofovir-emtricitabine, administered with efavirenz, in ART-naïve subjects. The primary renal results have been published elsewhere [27]. Here, we present the results of the powered secondary end point, change in BMD, over a 48-week period.

PATIENTS AND METHODS

This was a multicenter, randomized, open-label, study that was conducted in 76 centers across 13 countries in Europe. The study design and patient population have been described previously [27]. In brief, eligible ART-naïve, *HLA-B*5701*-negative, HIV-infected adult subjects were randomized to receive either abacavir-lamivudine or tenofovir-emtricitabine, with efavirenz, for 96 weeks, with the primary analyses conducted after 48 weeks of treatment. Randomization was stratified by screening estimated glomerular filtration rate (eGFR), Black or non-Black race, and body mass index (BMI, calculated as the weight in

Table 1. Demographic and Baseline Characteristics of the Intent-to-Treat Exposed Population

Variable	ABC-3TC (n = 192)	TDF-FTC (n = 193)	Total (n = 385)
Age, median years (range)	38.0 (19–70)	36.0 (18–66)	37.0 (18–70)
Male sex	159 (83)	154 (80)	313 (81)
Race			
Black ^a	26 (14)	30 (16)	56 (15)
White	152 (79)	150 (78)	302 (78)
Other	14 (7)	13 (7)	27 (7)
Weight, median kg (range)	71.0 (46.0–175.0)	73.0 (33.0–107.0)	72.0 (33.0–175.0)
Current smoker	69 (36)	74 (38)	143 (37)
History of fracture			
Nonvertebral	24 (13)	29 (15)	53 (14)
Vertebral	2 (1)	1 (<1)	3 (<1)
Z-score, mean value (±SD) ^b			
Hip	−0.25 (0.90)	−0.21 (0.94)	ND
Spine	−0.53 (1.31)	−0.44 (1.2)	ND
Hepatitis C reactive	16 (8)	18 (9)	34 (9)
Screen estimated glomerular filtration rate ^a			
<90 mL/min/1.73m ²	62 (32)	63 (33)	125 (32)
≥90 mL/min/1.73m ²	130 (68)	130 (67)	260 (68)
Baseline BMI ^a			
<25	127 (66)	130 (67)	257 (67)
≥25	64 (33)	63 (33)	127 (33)
Missing	1 (<1)	0	1 (<1)
Baseline CD4 ⁺ cell count, median cells/mm ³ (range)	240.0 (10–600)	230.0 (10–600)	240.0 (10–610)
Baseline HIV RNA level, median plasma HIV RNA log ₁₀ copies/mL (range)	5.01 (2.88–6.78)	5.12 (3.31–6.75)	5.06 (2.88–6.78)
CDC category C (AIDS)	10 (5)	18 (9)	28 (7)

NOTE. Data are no. (%) of subjects, unless otherwise indicated. ABC-3TC, abacavir-lamivudine fixed dose combination daily plus efavirenz; BMI, body mass index, calculated as weight in kilograms divided by the square of height in meters; CDC, Centers for Disease Control and Prevention; HIV, human immunodeficiency virus; ND, not done; TDF-FTC, tenofovir-emtricitabine fixed dose combination daily plus efavirenz.

^a Randomization strata.

^b Bone mineral density data was not available for all patients at baseline. At the hip, *n* = 176 in the abacavir-lamivudine arm and *n* = 180 in the tenofovir-emtricitabine arm. At the spine, *n* = 181 in the abacavir-lamivudine arm and *n* = 183 for tenofovir.

kilograms divided by the square of height in meters). Drugs known to affect GFR or BMD, including oral glucocorticoids, calcium, and vitamin D supplements, were prohibited in the protocol.

Dual energy x-ray absorptiometry (DXA) scans (of the lumbar spine and hip) were conducted at baseline, week 24, and week 48 (plus the withdrawal visit, if applicable). These were read centrally by CCBR-Synarc (Hamburg, Germany), with investigators and subjects remaining blinded to the results. Patients were scanned with the same machine throughout the course of the study, and phantom scans were provided to Synarc for quality assurance.

If a subject experienced a confirmed decrease in BMD of >6% (outside the least significant change, as defined by the International Society for Clinical Densitometry [28]), the investigator was unblinded to the subject's full BMD results. The subject could discontinue the study or continue in the study

with calcium and vitamin D supplements as necessary. Subjects who received tenofovir-emtricitabine could switch to abacavir-lamivudine. Any post-switch data were excluded from analyses.

Serum and plasma samples were collected at baseline and at weeks 12, 24, and 48. Osteocalcin (plasma) was assayed by an Electroluminescence N-MID method (Elecsys Systems; Hoffmann–La Roche) (normal range, 8.0–37.0 µg/L for men and 7.0–38.0 µg/L for women without osteoporosis). BSAP (in serum) was assayed by a chemiluminescent immunoassay (Access Immunoassay System; Beckman Coulter) (normal range, 3.7–29.9 µg/L for men, 2.9–14.5 µg/L for pre-menopausal women, and 3.8–22.6 µg/L for post-menopausal women). P1NP (in plasma) was assayed by a radioimmunoassay (UniQ P1NP RIA kit; Orion Diagnostica) (normal range, 22–87 µg/L for men, 19–83 µg/L for pre-menopausal women, and 16–96 µg/L for post-menopausal women). CTx (in serum) was assayed by enzyme-linked immunosorbent assay (ELISA) (normal range,

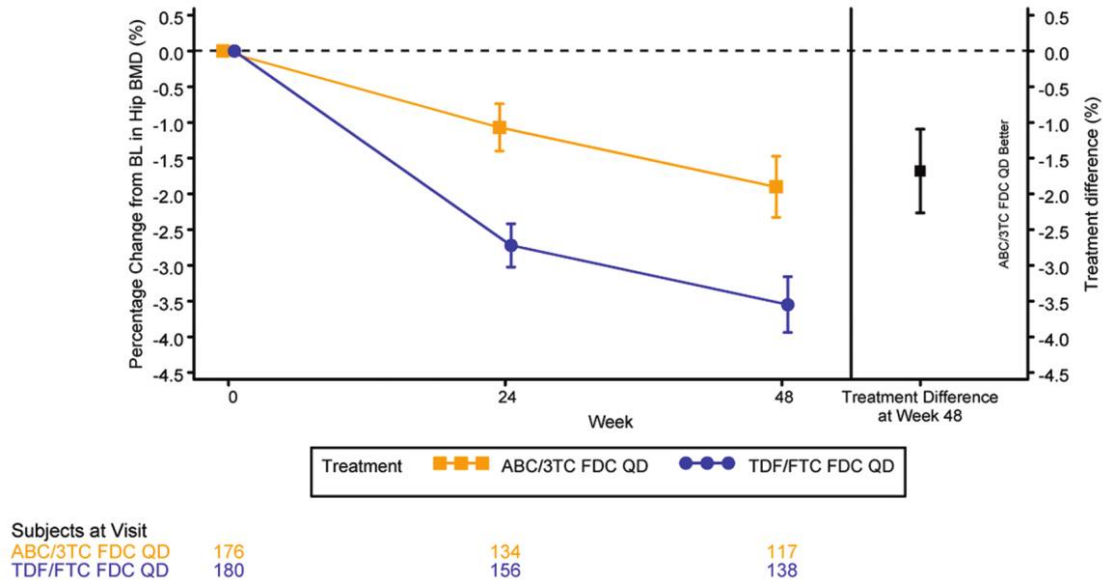


Figure 2. Adjusted mean percentage change from baseline (BL) in total hip bone mineral density (BMD) (%) by visit, with use of repeated-measures mixed-model analysis. Multivariate analysis was adjusted for the following covariates: visit, baseline hip BMD, baseline body mass index (BMI), calculated as the weight in kilograms divided by the square of height in meters), race, risk factor, prohibited medication, history of fracture, treatment by visit interaction, baseline hip BMD by visit interaction, and baseline BMI by visit interaction. ABC, abacavir; FDC, fixed drug combination; FTC, emtricitabine; QD, once daily; TDF, tenofovir; 3TC, lamivudine.

≤915 ng/L). Bone biomarker assays were conducted by Quest Diagnostics in batches throughout the study. Coefficients of variation were within accepted standard limits. β_2 -microglobulin (B2M) and retinol binding protein (RBP) were analyzed as described elsewhere [27].

The protocol outlined power calculations for the key secondary end points: change from baseline to week 48 in total hip and lumbar spine BMD. It was calculated that, if at least 107 of the 190 subjects per arm had DXA performed, this would be sufficient to detect a 2% difference between the treatment groups (assuming a 4.5% standard deviation) with 90% power at the 2-sided 5% significance level for both sites. The difference between treatment groups in total hip and lumbar spine BMD was assessed using a repeated measures mixed model analysis with adjustment for influential baseline covariates (namely, baseline BMD, the randomization strata race and baseline BMI, age, history of previous fractures, and use of prohibited medications). Other baseline factors were explored but were not retained in either of the final analysis models for hip or spine BMD because they were not influential; interactions of interest were also explored. The proportion of subjects with a decrease in BMD of $\geq 6.0\%$ was summarized. Mean Z-scores were calculated in a post-hoc analysis. The differences between the groups with respect to the changes from baseline to week 24 and week 48 in bone turnover markers were assessed using analysis of variance models, adjusting for baseline marker level, age, sex, race, baseline viral load, and baseline CD4⁺ cell count,

as required. P1NP bone marker data were not normally distributed and therefore were log transformed prior to analysis.

BMD analyses were conducted on the intent-to-treat exposed (ITT-E) population, comprising all randomized subjects who received ≥ 1 dose of study medication, using an observed case dataset in which only on-treatment data were included, with no imputations for missing data or early discontinuation of therapy. Bone turnover marker analyses were conducted on the safety biomarker population, which included those members of the ITT-E population who had results for at least 1 bone turnover marker at baseline and 1 time point after baseline.

RESULTS

In total, 392 subjects were randomized into the study, and 385 subjects received treatment with either abacavir-lamivudine (192 subjects) or tenofovir-emtricitabine (193 subjects). At week 48, 107 subjects (28%) had withdrawn prematurely from the study, including 63 subjects (33%) who received abacavir-lamivudine and 44 subjects (23%) who received tenofovir-emtricitabine (Figure 1). The treatment groups were well matched for demographic and baseline characteristics (Table 1), but some differences were observed with respect to the primary reason for withdrawal.

At baseline, there was no apparent association between BMD and markers of disease severity, such as viral load and CD4⁺

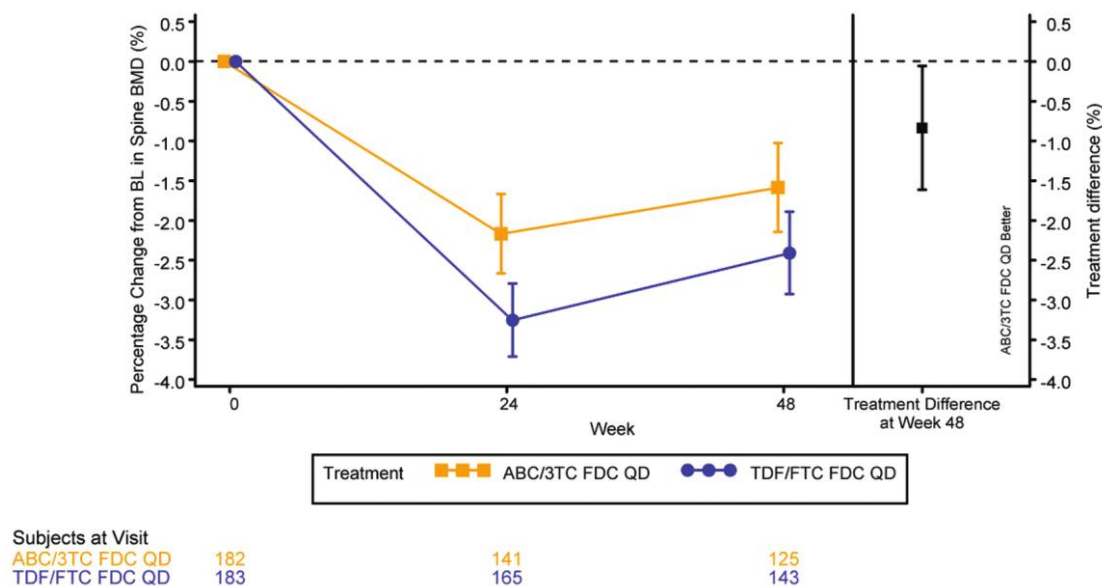


Figure 3. Adjusted mean percentage change from baseline (BL) in lumbar spine bone mineral density (BMD) (%) by visit, with use of a repeated-measures mixed-model analysis. Multivariate analysis was adjusted for the following covariates: visit, baseline hip BMD, baseline body mass index (BMI, calculated as the weight in kilograms divided by the square of height in meters), race, age group, treatment by visit interaction, baseline hip BMD by visit interaction, and baseline BMI by visit interaction. ABC, abacavir; FDC, fixed drug combination; FTC, emtricitabine; QD, once daily; TDF, tenofovir; 3TC, lamivudine.

cell count; only older age (>45 years) appeared to be associated with lower baseline BMD, as would be expected.

Percentage BMD change from baseline. Of the 385 ITT-E subjects, 365 had a hip and/or spine BMD measurement at baseline. At week 48, a decrease in BMD was observed in each treatment group in both total hip and lumbar spine BMD. The adjusted mean percentage change from baseline in total hip BMD was -1.9% in the abacavir-lamivudine group and -3.6% in the tenofovir-emtricitabine group (treatment difference -1.7% (95% confidence interval [CI], -2.26 to -1.10 ; $P < .001$) (Figure 2). The decrease in the abacavir-lamivudine

group was maintained at a constant rate throughout the 48 weeks, whereas in the tenofovir-emtricitabine group, BMD decreased steeply over the initial 24-week period, then continued to decrease at a rate similar to that in the abacavir-lamivudine group.

Of those covariates adjusted for in the analysis, lower baseline BMI, non-Black race, use of prohibited medications, and lack of previous fractures were significantly ($P < .03$) associated with greater bone loss in total hip BMD.

The adjusted mean percentage change from baseline in lumbar spine BMD was -1.6% in the abacavir-lamivudine group

Table 2. Proportion of Subjects with Decrease from Baseline Total Hip and Lumbar Spine Bone Mineral Density (BMD): Observed Analysis of the Intent-to-Treat Exposed Population

Variable	ABC-3TC (n = 192)		TDF-FTC (n = 193)	
	No. of subjects	No. (%) of subjects with decrease in BMD $\geq 6\%$	No. of subjects	No. (%) of subjects with decrease in BMD $\geq 6\%$
Total hip, actual relative time				
Week 24	137	1 (<1)	160	6 (4)
Week 48	120	3 (3)	143	18 (13)
Lumbar spin, actual relative time				
Week 24	142	10 (7)	165	17 (10)
Week 48	126	6 (5)	143	15 (10)

NOTE. ABC-3TC, abacavir-lamivudine fixed-dose combination daily plus efavirenz; TDF-FTC, tenofovir-emtricitabine fixed dose combination daily plus efavirenz.

Table 3. Change from Baseline to Week 48 in Bone Biomarkers

Bone biomarker, treatment group	No. of subjects	Week 48 value as a percentage of baseline, %	Ratio of change from baseline (95% CI)	Mean change from baseline	Mean difference (95% CI)	<i>P</i>
P1NP, mcg/L			1.3 (1.2–1.4)			<.001
ABC-3TC	114	130		
TDF-FTC	134	160		
CTx, ng/L					64.3 (–28.0 to 156.7)	.172
ABC-3TC	112	+206.7		
TDF-FTC	130	+271.0		
Osteocalcin, mg/L					3.85 (2.10–5.60)	<.001
ABC-3TC	114	+8.07		
TDF-FTC	134	+11.92		
Bone-specific alkaline phosphatase, mg/L					3.31 (1.82–4.81)	<.001
ABC-3TC	113	+4.34		
TDF-FTC	134	+7.65		

NOTE. P1NP data were log transformed prior to analysis, and results are given as percentage changes from baseline, ABC-3TC, abacavir-lamivudine fixed-dose combination daily plus efavirenz; CI, confidence interval; TDF-FTC, tenofovir-emtricitabine fixed-dose combination daily plus efavirenz.

and –2.4% in the tenofovir-emtricitabine group (treatment difference, –0.8%; 95% CI, –1.61% though –0.06%; *P* = .036) (Figure 3). For both treatment groups, the steepest decrease in BMD was observed in the first 24 weeks of treatment, with some recovery between weeks 24 and 48.

Of those covariates that were adjusted for in the analysis, lower baseline BMI, non-Black race, and older age (≥ 45 years) were significantly (*P* < .03) associated with a greater percentage decrease in lumbar spine BMD.

Greater than 6% decrease in BMD from baseline. At week 48, a greater proportion of subjects in the tenofovir-emtricitabine group had a decrease from baseline in both total hip and lumbar spine BMD of $\geq 6\%$ (hip, 13%; spine, 10%), compared with the abacavir-lamivudine group (hip, 3%; spine, 5%) (Table 2). There were 50 subjects with a reported BMD loss of $\geq 6\%$ at least once in the study: 13 subjects in the abacavir-lamivudine group (hip, 4 subjects; spine, 13 subjects), and 37 subjects in the tenofovir-emtricitabine group (hip, 21 subjects; spine, 23 subjects).

An investigation of subjects with $\geq 6\%$ bone loss at any point during the study indicated that this was more commonly reported by non-Black subjects than by Black subjects and more commonly reported by subjects with lower baseline BMI than by those with higher baseline BMI. There were no clear relationships between $\geq 6\%$ bone loss and sex, age group, smoking status, hepatitis C at baseline, or baseline GFR. Additional analysis by treatment group showed that these relationships were similar in both treatment groups.

Z-scores. At baseline, the mean Z-scores were similar between the 2 treatment groups (Table 1). For those with Z-score measurements at week 48, both arms showed a small decrease in mean (\pm standard deviation [SD]) Z-score from baseline: -0.11 ± 0.16 and -0.11 ± 0.26 in the abacavir-lami-

vudine group for total hip and lumbar spine, respectively, and -0.24 ± 0.18 and -0.22 ± 0.33 in the tenofovir-emtricitabine group for total hip and lumbar spine, respectively.

Bone turnover markers. Increases from baseline in all measured biomarkers were seen after baseline. The levels were highest at week 24 and stabilized or decreased thereafter. Increases in all markers were significantly greater in the tenofovir-emtricitabine group than in the abacavir-lamivudine group at week 24. These increases remained significantly different between the treatment groups at week 48 for all but CTx (Table 3).

Although the mean values for the population remained within the normal ranges quoted for each marker at all time points, an increasing proportion of the study population had levels above the upper limit of normal over the course of the study (Figure 4).

The changes from baseline to week 48 for all bone turnover markers were significantly positively correlated with each other (correlation co-efficient [*r*], 0.265–0.564; *P* < .001) except for CTx and BSAP (*r* = 0.137; *P* = .32). These changes in biomarkers were significantly negatively correlated with change in BMD from baseline, in both total hip and lumbar spine (*r* = –0.185 to –0.402; *P* \leq .008 [except BSAP: lumbar spine, *r* = –0.157; *P* = .017] (Figure 5).

To examine whether renal tubular proteinuria was associated with an increase in bone turnover markers, further correlations with changes from baseline in B2M and RBP in the urine (corrected for creatinine) were conducted. No statistically significant associations were found.

DISCUSSION

The European AIDS Clinical Society has recently added information and guidelines on diagnosing, managing, and moni-

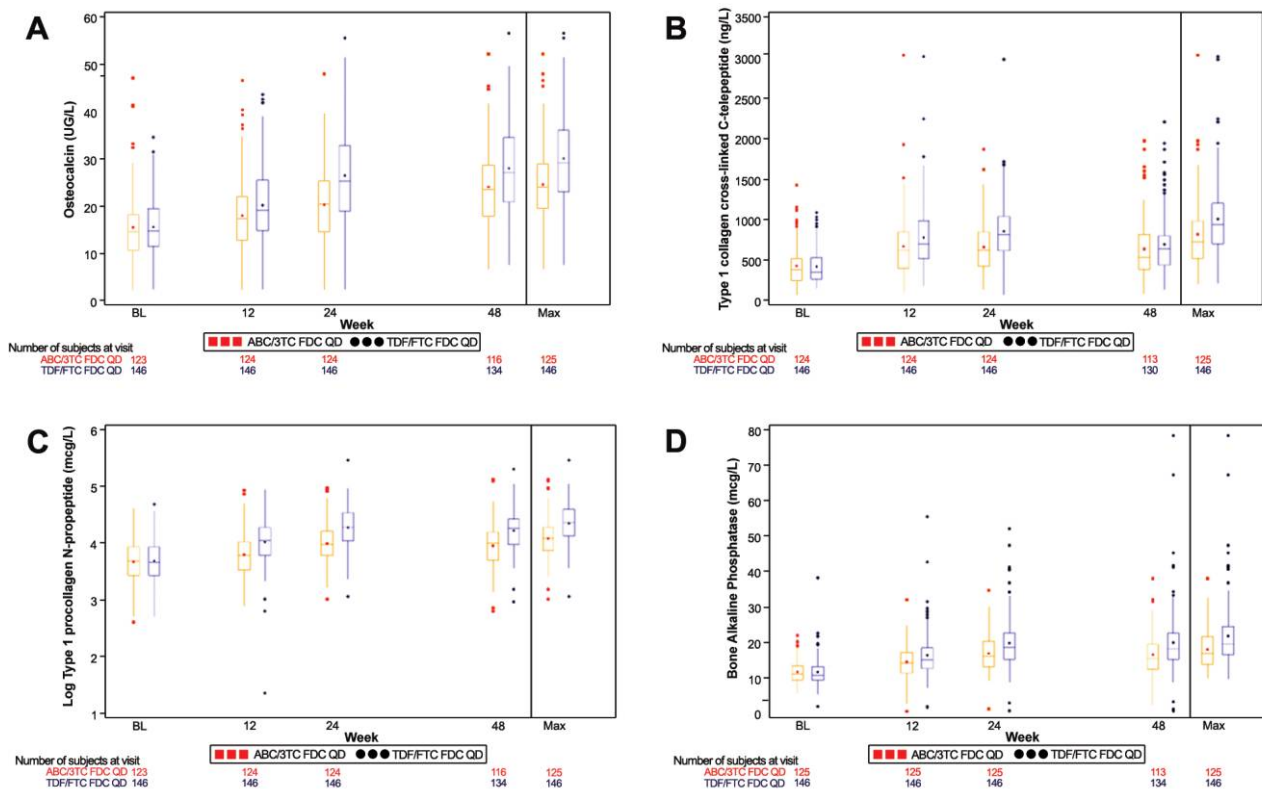


Figure 4. Box plot of bone biomarkers by treatment. *A*, Osteocalcin: normal range, 8.0–37.0 $\mu\text{g/L}$; lower limit of detection, 0.5 ng/L; coefficient of variance, 3.1%. *B*, CTx: normal range, ≤ 915 ng/L, 30 pg/mL, 12%. *C*, P1NP: normal range, 22–87 $\mu\text{g/L}$ (adult males), 19–83 $\mu\text{g/L}$ (pre-menopausal women), and 16–96 $\mu\text{g/L}$ (post-menopausal women), 5 ng/mL, 10%; *D*, BSAP: normal range, 3.7–29.9 $\mu\text{g/L}$ (adult males), 2.9–14.5 $\mu\text{g/L}$ (pre-menopausal women), and 3.8–22.6 $\mu\text{g/L}$ (post-menopausal women); lower limit of detection, 0.1 mcg/L; coefficient of variance, 8.3%. ABC, abacavir; BSAP, bone-specific alkaline phosphatase; CTx, type 1 collagen cross-linked C telopeptide; FDC, fixed drug combination; FTC, emtricitabine; P1NP, procollagen type 1 N-terminal propeptide; QD, once daily; TDF, tenofovir; 3TC, lamivudine.

toring bone disease in the HIV-infected population [1], emphasizing the importance of the impact of HIV infection and ART on bone. In this study, we sought to investigate the impact of initiating ART treatment with either abacavir-lamivudine or tenofovir-emtricitabine, both in combination with efavirenz, on bone.

The study demonstrated BMD loss in both treatment groups, with a significantly greater BMD loss in the tenofovir-emtricitabine group than in the abacavir-lamivudine group over a 48-week period. Similarly, the percentage of subjects who lost $\geq 6\%$ of their BMD within 1 year was greater in the tenofovir-emtricitabine group than in the abacavir-lamivudine group. The fact that changes observed between weeks 24 and 48 differed for the total hip and lumbar spine measurements indicates different kinetics of response to ART at different anatomical sites. The difference between the treatment groups, however, remained statistically significant at both time points. Because T-scores are only applicable to post-menopausal women and older men, we calculated Z-scores for our population. The mean Z-scores were similar between the arms and decreased

slightly over a 48-week period, suggesting that our population was losing BMD at a rate similar to that for the non-HIV-infected population of the same age.

A decrease in BMD was observed in both groups, suggesting that there is at least a transient treatment effect on bone remodeling with both regimens. Loss of BMD has been reported among HIV-infected patients who initiated ART (specifically, within the first 24–48 weeks of ART), irrespective of the regimen used [13, 17, 29], although initial changes may stabilize after 1 year of treatment [9, 10, 15]. This suggests that initial losses of BMD may be attributable to the continued impact of HIV infection during the period in which it is brought under control. However the Strategies for Management of Antiretroviral Therapy (SMART) study showed a significantly greater loss of BMD among patients who received continuous ART than among patients who received intermittent ART [29], and the Simplification with Fixed Dose Tenofovir-Emtricitabine or Abacavir-Lamivudine in Adults with Suppressed HIV Replication (STEAL) study showed changes in BMD in patients with virologically controlled infection who switched therapy, with

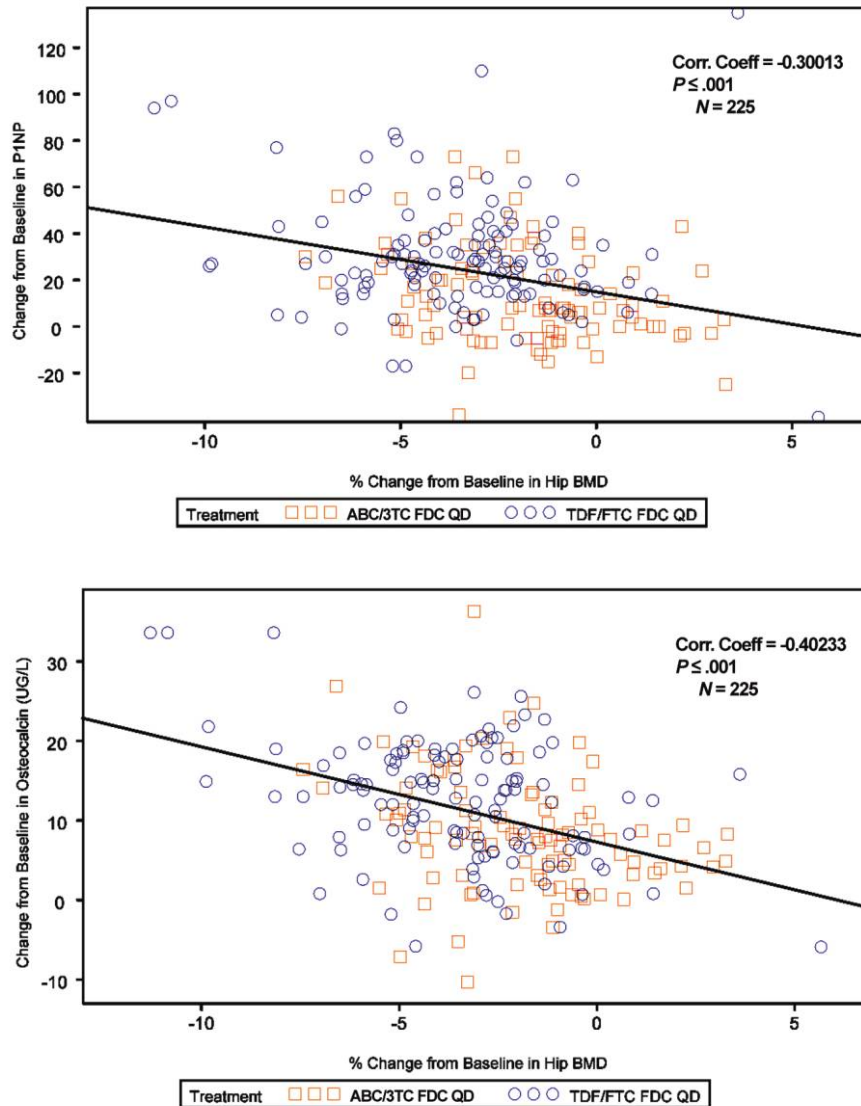


Figure 5. Scatter plot of change from baseline to week 48 versus change from baseline in total hip P1NP (A) and osteocalcin (B). ABC, abacavir; BMD, bone mineral density; FDC, fixed drug combination; FTC, emtricitabine; P1NP, procollagen type 1 N-terminal propeptide; QD, once daily; TDF, tenofovir; 3TC, lamivudine.

an increase of BMD in the abacavir-lamivudine arm and a loss of BMD in the tenofovir-emtricitabine arm [10]. These studies suggest that ART itself does have an impact on BMD and that different regimens can impact bone differently.

When our study was designed, protease inhibitors were reported to be associated with greater loss of BMD [14, 15, 30], whereas efavirenz was thought to have little impact on the bone end points. Efavirenz was, therefore, chosen as the third agent in this study. Subsequent studies that investigated BMD among patients who received efavirenz have suggested that efavirenz may also adversely affect BMD [22, 23, 31]. This raises the possibility that efavirenz could also be impacting the BMD

results presented here, although we have no way of determining to what degree it may do so.

In the general population, BMD increases in both men and women throughout childhood and puberty, reaching a stable peak at 25–35 years of age [32]. Thereafter, BMD decreases slowly, with accelerated loss in peri- and post-menopausal women [32, 33]. It has been reported that post-menopausal women may lose 1.0%–1.4% BMD per year in the hip and 0.13%–2.48% per year in the spine, with weight and ethnicity being strong modifiers of the rate of BMD loss [33, 34]. In HIV-infected patients, rates of BMD decrease of up to 0.8% per year for men and pre-menopausal women, with or without

ART, have been reported [29, 35, 36], although the rate of BMD loss is dynamic over time. Our results confirm that patients can have significant BMD loss within the first year of ART. Such significant BMD loss is particularly concerning, because our population was largely composed of young men, whose BMD should be relatively stable. In females with a history of fracture and those taking little physical exercise, any loss of BMD could become a clinically relevant problem during long-term survival. As the age of the HIV-infected population increases, more patients will become osteoporotic and at risk of fracture. Longer follow-up is needed to assess the course of BMD loss over time, as well as the clinical relevance of BMD loss in the HIV-infected population.

We also investigated the impact of the 2 treatment regimens on 3 markers of bone formation and 1 marker of bone resorption, all of which initially increased in both groups and then stabilized or decreased. These markers reflect whole-body bone turnover and provide information about the mechanisms underlying changes in BMD. The increases observed in both treatment groups after the initiation of therapy indicate that bone loss was attributable to an increased remodeling rate, with resorption and formation remaining coupled. The significant correlations between changes in bone turnover markers and BMD are consistent with this proposed mechanism of bone loss. A significantly greater increase from baseline was observed in the tenofovir-emtricitabine group in all markers at week 24, and this difference remained significant for all markers except CTx at week 48. The stabilization of bone biomarkers between weeks 24 and 48, mostly at an increased rate from baseline, indicates that a new steady state of bone remodeling was reached while receiving ART. We chose to concentrate on bone turnover markers in this study, and a limitation of our work is that we did not assess vitamin D status.

In post-menopausal women, bone turnover markers also correlate negatively with BMD ($r = -0.35$ to -0.4) regardless of skeletal site, indicating the role played by high bone turnover in BMD loss [25]. Although bone biomarkers may provide additional information to supplement BMD measurements, they have limited value in identifying those who are fast bone losers [25], because of the larger range of individual values. Here, we found no correlation between B2M or RBP and changes in the bone turnover markers, which suggest that the bone loss was not caused by renal dysfunction. However, we did not measure phosphate loss from the kidney, which would have been a more useful marker to evaluate any link between bone loss and kidney dysfunction.

Initiation of ART is associated with increased bone turnover and bone loss from the spine and hip, with a number of subjects losing $\geq 6\%$ BMD within 1 year after starting treatment. These effects were significantly greater among those who received the tenofovir-containing regimen, but the clinical significance over

the longer term remains to be determined. The continuation of the study to 96 weeks will provide an opportunity to investigate this.

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