Bone Mineral Density and Fractures in Antiretroviral-Naive Persons Randomized to Receive Abacavir-Lamivudine or Tenofovir Disoproxil Fumarate-Emtricitabine Along With Efavirenz or Atazanavir-Ritonavir: AIDS Clinical Trials Group A5224s, a Substudy of ACTG A5202

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(See the editorial commentary by Yin and Overton, on pages 1705–7.)

Background. Long-term effects of abacavir (ABC)–lamivudine (3TC), compared with tenofovir (TDF)–emtricitabine (FTC) with efavirenz (EFV) or atazanavir plus ritonavir (ATV/r), on bone mineral density (BMD) have not been analyzed.

Methods. A5224s was a substudy of A5202, in which HIV-infected treatment-naive participants were randomized and blinded to receive ABC-3TC or TDF-FTC with open-label EFV or ATV/r. Primary bone end points included Dual-emission X-ray absorbtiometry (DXA)-measured percent changes in spine and hip BMD at week 96. Primary analyses were intent-to-treat. Statistical tests used the factorial design and included linear regression, 2-sample *t*, log-rank, and Fisher's exact tests.

Results. Two hundred sixty-nine persons randomized to 4 arms of ABC-3TC or TDF-FTC with EFV or ATV/r. At baseline, 85% were male, and 47% were white non-Hispanic; the median HIV-1 RNA load was 4.6 $\log_{10}$ copies/mL, the median age was 38 years, the median weight was 76 kg, and the median CD4 cell count was 233 cells/μL. At week 96, the mean percentage changes from baseline in spine and hip BMD for ABC-3TC versus TDF-FTC were -1.3% and -3.3% ($P = .004$) and -2.6% and -4.0% ($P = .024$), respectively; and for EFV versus ATV/r were -1.7% and -3.1% ($P = .035$) and -3.1% and -3.4% ($P = .61$), respectively. Bone fracture was observed in 5.6% of participants. The probability of bone fractures and time to first fracture were not different across components.

Conclusions. Compared with ABC-3TC, TDF-FTC–treated participants had significantly greater decreases in spine and hip BMD, whereas ATV/r led to more significant losses in spine, but not hip, BMD than EFV.

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With the advent of potent antiretroviral therapy (ART), significant comorbidities have emerged, including osteoporosis and increased risk of fractures. Low bone mineral density (BMD) has been reported in studies of HIV-infected individuals; in a meta-analysis, the prevalence of osteoporosis was 3 times higher in HIV-infected patients than HIV-uninfected control subjects [1]. Studies have shown that BMD decreases by 2%–6% within the first 2 years of ART initiation, regardless of the choice of therapy [2–5], with a long-term study showing that this initial decrease is not progressive [3]. Studies reporting increased fracture rates in HIV-infected individuals are emerging [6–9].

Treatment with the nucleotide analogue reverse-transcriptase inhibitor tenofovir disoproxil fumarate (TDF) has been associated with an initial decrease in BMD [2]. In addition, there was more bone loss in virologically suppressed persons who switched to TDF, compared with switching to the nucleoside analogue reverse-transcriptase inhibitor (NRTI) abacavir (ABC) [10]. To date, there has been a single report of a 48-week prospective study of participants initiating their first ART with TDF-emtricitabine (FTC) or ABC-lamivudine (3TC), combined with the nonnucleoside reverse-transcriptase inhibitor (NNRTI) efavirenz (EFV) [11]. A significantly greater decrease in spine and hip BMD was seen with TDF-FTC. To date, there has been no study comparing the effects on bone of EFV compared with those of atazanavir-ritonavir (ATV/r), a protease inhibitor (PI) combination with few metabolic effects [12, 13].

METHODS

A5224s was a substudy of AIDS Clinical Trials Group (ACTG) A5202, in which ART-naive persons aged $\geq$16 years and with an HIV-1 RNA load >1000 copies/mL were randomized in a double-blinded fashion to receive coformulated TDF-FTC or ABC-3TC, along with open-labeled EFV or ATV/r at standard doses. A coprimary objective of A5224s was to compare the effects of initiating ABC-3TC with those of TDF-FTC on spine and hip BMD. The second coprimary objective was to assess the effect of these drugs on body fat; results of these analyses will be reported elsewhere. A5224s secondary objectives were to compare BMD changes between EFV and ATV/r arms, to compare TDF-FTC with ABC-3TC and EFV with ATV/r on BMD changes at week 48, and to compare the proportion of participants with bone fractures during study. Specific A5224s exclusion criteria were uncontrolled thyroid disease or hypogonadism; endocrine diseases, including Cushing's syndrome, diabetes mellitus, and the use of growth hormone, anabolic steroids, glucocorticoids, or osteoporosis medications; or the intent to start bone-related treatment. The duration of the study was 96 weeks after the last participant enrolled.

Any participant enrolling in A5202 at one of ACTG sites participating in A5224s and meeting criteria for A5224s was eligible to enroll. Each participant signed a written informed consent before enrollment. The study was approved by the local institutional review board at each site.

At baseline, a complete history, including history of fractures, was obtained, and participants underwent a physical examination, including measurement of height and weight. Substudy evaluation included BMD measurement by dual-energy absorptiometry (DXA) in the anteroposterior view (using Hologic or Lunar scanners) of the lumbar spine (from L1-L4) and hip at baseline and at weeks 24, 48, 96, 144, and 192. To assess for osteopenia at baseline, we used *t* scores (standard deviations from the mean value in young normal individuals) at the spine or hip, based on the manufacturers' sex- and ethnicity-specific reference populations. Technicians were instructed to scan the same hip of each participant and use the same machine on the same participant throughout the study. All DXAs were standardized at the participating sites, then centrally read (Tufts) by blinded personnel. On 18 February 2008 [14], the parent study A5202 team was notified of the Data Safety and Monitoring Board (DSMB) recommendation to unblind the NRTI assignment for participants with screening HIV-1 RNA loads $\geq$100,000 copies/mL because of excess virologic failures seen in this subgroup who were receiving ABC-3TC regimens.

Statistical Analysis

The primary DXA objectives were to compare, between pooled, randomized NRTI components (ABC-3TC vs TDF-FTC), changes from baseline to week 96 in spine and hip BMD. Other objectives and analyses were considered to be secondary. A5224s was originally powered as a factorial analysis. With a sample size of 125 participants per component, there was 98% power to detect the prespecified 2% between 2 groups difference in BMD percentage change.

All analyses were initially performed using intent-to-treat (ITT) principles based on randomized treatment assignment in which all available data were used and modifications to randomized treatment and missing values were ignored. Supplemental as-treated (AT) analyses were performed in which values were censored after a change in the randomized NRTI component (when comparing NRTI components) or NNRTI/PI component (when comparing NNRTI/PI components). *P* values <.05 were interpreted as statistically significant, and nominal values are reported without adjustment for multiple comparisons. Analyses were performed using SAS, version 9.1.3 (SAS Institute).

Comparisons between regimen components used 2-sample *t*, Fisher's exact, or log-rank tests, as appropriate. Analyses that adjusted for baseline factors and explored associations with baseline factors used linear regression. Mixed model analysis of variance with an unstructured correlation structure was used to

test for differences in change from baseline between components over time. Time was modeled using piecewise variables, in which one variable captured the linear slope for changes from baseline to week 48 and the second variable captured the linear slope for changes from week 48 to 192. The week-48 separation time point was chosen on the basis of consultation with study chairs, after visual inspection of the data.

RESULTS

Participant Characteristics

A total of 271 participants from 37 ACTG sites in the United States and Puerto Rico intended to participate in A5224s and were randomized to receive ART; of these, 2 were excluded from the analysis when found to have had an eligibility violation. Enrollment spanned from 5 October 2005 through 7 November 2007 with 69 participants randomized to receive EFV plus TDF-FTC, 70 to EFV plus ABC-3TC, 65 to ATV/r plus TDF-FTC, and 65 to ATV/r plus ABC-3TC. Baseline characteristics are summarized in Table 1. Overall, 85% of participants were male and 47% were non-Hispanic white persons. The median age was 38 years, body mass index (BMI; measured as the weight in kilograms divided by the square of the height in meters) was 24.9, CD4 cell count was 233 cells/μL, and HIV-1 RNA load was 4.62 $\log_{10}$ copies/mL. One hundred sixty participants (59%) enrolled had an HIV-1 RNA load <100,000 copies/mL at study screening. Overall, 3% were hepatitis B surface antigen positive, 9% was hepatitis C antibody positive, 32% reported a history of fracture, and 39% had osteopenia (*t* score ≤-1 at spine or hip) at study entry. The baseline characteristics were balanced across arms.

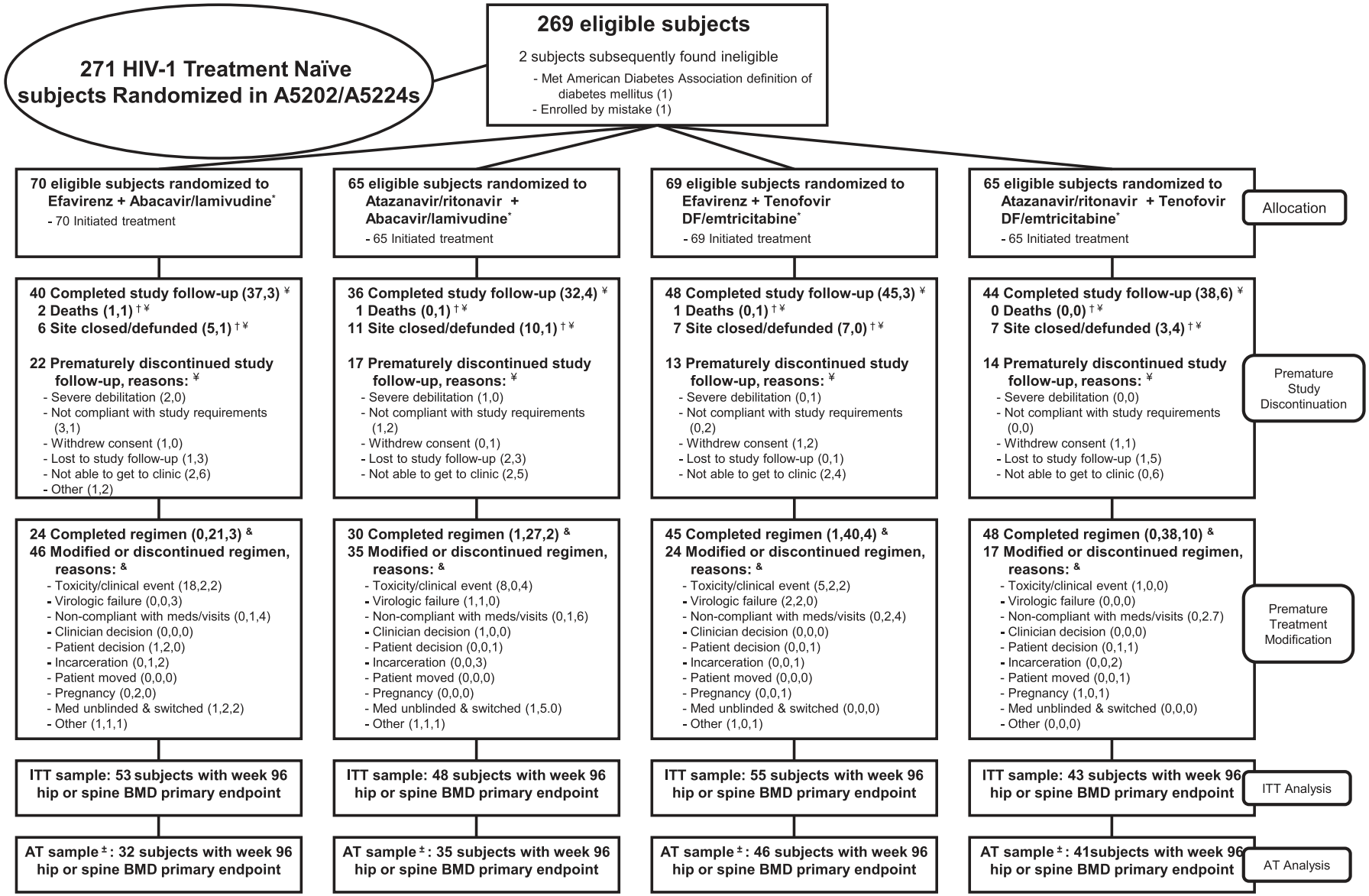
The baseline characteristics of the A5224s participants were compared with those of the 1588 A5202 persons who did not participate in the substudy; no statistically significant differences were found for age, BMI, CD4 cell count, HIV-1 RNA load, or history of fractures. However the non-A5224s group included significantly more Hispanic persons (24% vs 16%; $P = .005$).

Participant Disposition

Figure 1 details the disposition of all participants. Overall, 66 (25%) of the A5224s participants prematurely discontinued the substudy, and 4 (1%) died. In addition, 31 participants (12%)

Table 1. Baseline Characteristics of Study Participants, by Randomized Arms

Characteristic		EFV + TDF-FTC (*n* = 69)	EFV + ABC-3TC (*n* = 70)	ATV/r + TDF-FTC (*n* = 65)	ATV/r + ABC-3TC (*n* = 65)	Total (*n* = 269)
Age (years)	Median (Q1-Q3)	40 (33-44)	39 (31-46)	38 (30-44)	37 (29-43)	38 (31-44)
Sex	Male	58 (84%)	56 (80%)	56 (86%)	59 (91%)	229 (85%)
	Female	11 (16%)	14 (20%)	9 (14%)	6 (9%)	40 (15%)
Race/Ethnicity	White non-Hispanic	37 (54%)	34 (49%)	26 (40%)	29 (45%)	126 (47%)
	Black non-Hispanic	22 (32%)	20 (29%)	21 (32%)	27 (42%)	90 (33%)
	Hispanic (regardless of race)	8 (12%)	14 (20%)	14 (22%)	8 (12%)	44 (16%)
	Other	2 (3%)	2 (3%)	4 (6%)	1 (2%)	9 (3%)
BMI (kg/m^2)	Median (Q1-Q3)	24.9 (21.6-27.1)	24.7 (22.6-28.3)	24.9 (21.8-28.8)	25.3 (21.8-28.9)	24.9 (21.8-28.2)
CD4 category (cells/μL)	Median (Q1-Q3)	250 (132-334)	213 (106-350)	247 (114-319)	222 (75-332)	233 (106-334)
HIV-1 RNA ($\log_{10}$ copies/mL)	Median (Q1-Q3)	4.7 (4.2-4.9)	4.7 (4.2-4.9)	4.5 (4.2-4.9)	4.6 (4.3-5.1)	4.6 (4.2-4.9)
HIV-1 RNA (copies/mL)	< 100,000 copies/mL	56 (81%)	59 (84%)	52 (80%)	48 (74%)	215 (80%)
	≥ 100,000 copies/mL	13 (19%)	11 (16%)	13 (20%)	17 (26%)	54 (20%)
History of bone fracture	Yes	22 (32%)	24 (34%)	18 (28%)	22 (34%)	86 (32%)
	No	47 (68%)	46 (66%)	46 (71%)	43 (66%)	182 (68%)
	Not Evaluated	0 (0%)	0 (0%)	1 (2%)	0 (0%)	1 (<1%)
Lumbar spine BMD (g/cm^2)	Median (Q1-Q3)	1.12 (1.00-1.23)	1.08 (.97-1.23)	1.13 (1.03-1.24)	1.13 (1.04-1.23)	1.12 (.99-1.23)
Hip BMD (g/cm^2)	Median (Q1-Q3)	0.99 (.92-1.07)	1.02 (.93-1.11)	1.05 (.98-1.18)	1.02 (.97-1.13)	1.02 (.94-1.11)
Lumbar spine t-score	> -1	45 (67%)	38 (58%)	39 (64%)	46 (72%)	168 (65%)
	> -2.5 to ≤ -1	19 (28%)	23 (35%)	18 (30%)	13 (20%)	73 (28%)
	≤ -2.5	3 (4%)	5 (8%)	4 (7%)	5 (8%)	17 (7%)
Hip t-score	> -1	50 (75%)	48 (75%)	47 (80%)	51 (80%)	196 (77%)
	> -2.5 to ≤ -1	17 (25%)	15 (23%)	11 (19%)	13 (20%)	56 (22%)
	≤ -2.5	0 (0%)	1 (2%)	1 (2%)	0 (0%)	2 (1%)



Note: subjects were to remain in follow-up regardless of having modified antiretroviral therapy.

* NRTIs were double-blinded through February 25, 2008 for those with screening HIV-1 RNA ≥100,000 copies/mL, and until final visits starting July 1, 2009 for those with screening RNA < 100,000 copies/mL.

¥ Reasons for study discontinuation are split into (# with, # without) week 96 hip or spine BMD primary endpoint.

† Site closure was censored for premature study and treatment discontinuation. Death was censored for premature study discontinuation and counted as reason for treatment discontinuation if there was no prior modification.

& Reasons for first treatment modification are split into (# before, # after, # without) week 96 hip or spine BMD primary endpoint.

± Subjects in the as-treated sample remained on randomized treatment through to the week 96 DXA.

Figure 1. Details of disposition and outcome of study participants.

Table 2. Percent Changes in Lumbar Spine and Hip BMD for All 4 Treatment Arms. The Duration of the Study Was 96 Weeks Since the Last Subject Enrolled, Thus the Smaller n in Later Time Points

		EFV + TDF/FTC (N=69)	EFV + ABC/3TC (N=70)	ATV/r + TDF/FTC (N=65)	ATV/r + ABC/3TC (N=65)	Total (N=269)
Change in lumbar spine BMD (%), week 0–24	N	57	60	54	62	233
	Mean (SD)	-3.28 (2.71)	-2.15 (2.69)	-4.40 (3.58)	-2.26 (3.28)	-2.98 (3.19)
week 0–48	N	56	53	49	53	211
	Mean (SD)	-3.46 (4.06)	-1.59 (4.42)	-4.23 (4.03)	-2.80 (4.20)	-3.00 (4.26)
	P value	<.001	.012	<.001	<.001	<.001
week 0–96	N	54	53	43	48	198
	Mean (SD)	-2.52 (4.08)	-.78 (5.20)	-4.38 (4.95)	-1.99 (4.69)	-2.33 (4.87)
	P value	<.001	.28	<.001	.005	<.001
week 0–144	N	46	40	41	40	167
	Mean (SD)	-2.60 (4.58)	0.12 (5.92)	-3.55 (5.25)	-1.67 (3.93)	-1.96 (5.10)
week 0–192	N	30	28	23	25	106
	Mean (SD)	-2.02 (3.92)	-0.37 (6.67)	-4.93 (5.76)	-1.15 (4.32)	-2.01 (5.45)
Change in hip BMD (%), week 0–24	N	57	57	52	62	228
	Mean (SD)	-3.18 (5.13)	-1.15 (2.58)	-2.41 (2.59)	-.90 (2.45)	-1.88 (3.49)
week 0–48	N	56	51	48	53	208
	Mean (SD)	-3.78 (3.63)	-2.46 (4.46)	-4.42 (3.21)	-2.69 (3.17)	-3.33 (3.71)
	P value	<.001	<.001	<.001	<.001	<.001
week 0–96	N	54	51	42	48	195
	Mean (SD)	-3.69 (3.81)	-2.54 (4.40)	-4.31 (5.17)	-2.68 (3.30)	-3.28 (4.22)
	P value	<.001	<.001	<.001	<.001	<.001
week 0–144	N	45	39	40	40	164
	Mean (SD)	-3.28 (3.74)	-2.71 (4.90)	-3.44 (5.63)	-2.79 (3.86)	-3.06 (4.54)
week 0–192	N	30	29	23	25	107
	Mean (SD)	-2.65 (4.17)	-2.34 (3.98)	-3.56 (6.04)	-1.47 (4.12)	-2.49 (4.57)

discontinued, because their sites were defunded during the study. There was no statistically significant difference in time to premature study discontinuation between NRTI components ($P = .13$, site closure and death censored) or NNRTI-PI components ($P = .86$). The median time from randomization to the last clinic visit was 165 weeks.

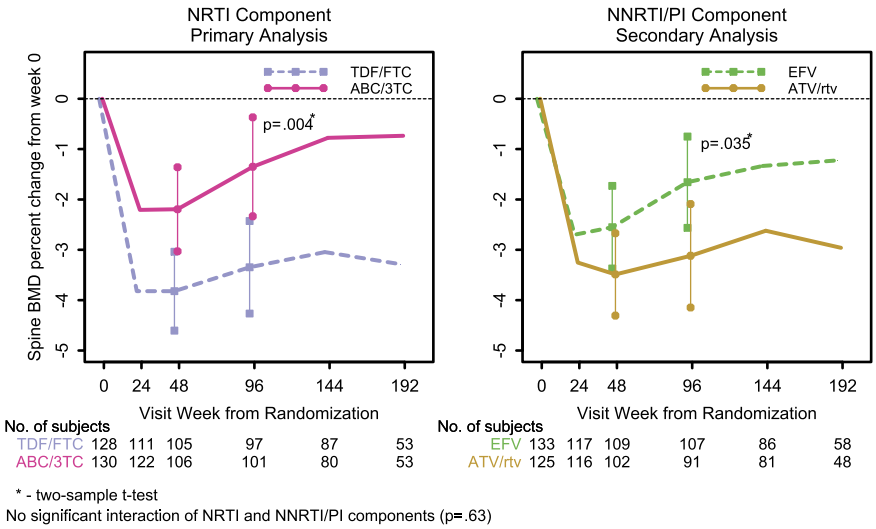


Figure 2. Mean percentage change in lumbar spine BMD by ITT analysis.

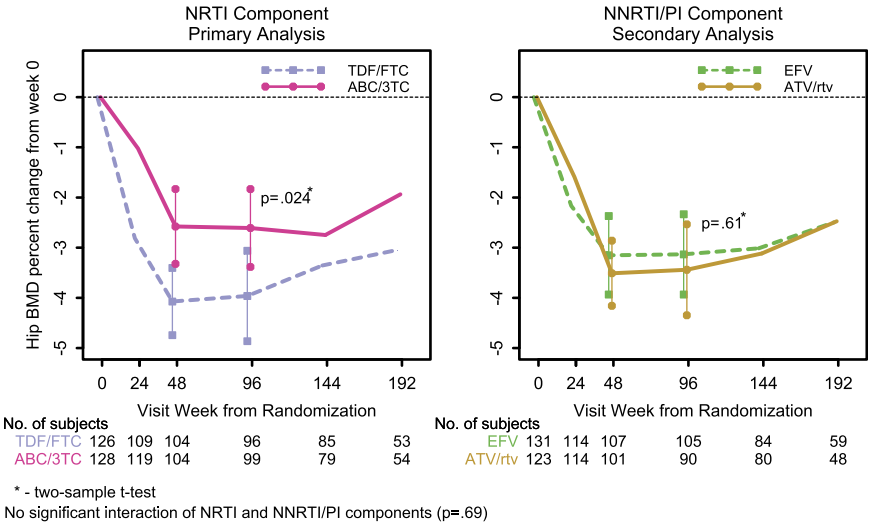


Figure 3. Mean percentage change in hip BMD by ITT analysis.

Percentage Changes in Spine BMD.

The first coprimary analysis assessed the difference in mean percentage change in spine BMD at week 96 between ABC-3TC and TDF-FTC. Table 2 summarizes the estimated mean percentage change over time in spine and hip BMD by all regimens. Figures 2 and 3 plot the mean percentage change over time in spine and hip BMD by NRTI and NNRTI-PI components.

The estimated mean percentage change in spine BMD for all participants was −3.0% at week 48 and −2.3% at week 96. The comparison of ABC-3TC (n = 135) and TDF-FTC (n = 134) with EFV and ATV/r combined (factorial analysis) was performed, because there was no significant evidence that the treatment effect between these drugs differed at 96 weeks by the NNRTI-PI component (P = .63). Similarly, the comparison of EFV (n = 139) and ATV/r (n = 130) with ABC-3TC and TDF-FTC combined was performed.

Changes by NRTI Components: Primary Analysis. By ITT at week 96, there was a significant decrease in mean percentage change in spine BMD for all arms except ABC-3TC plus EFV, but significantly less for ABC-3TC (estimated mean of −1.3%) than for TDF-FTC (−3.3%; difference [Δ] = 2.0%; 95% confidence interval [CI], .7%–3.3%; P = .004). The AT analysis showed similar results, with the mean percentage change in ABC-3TC– and TDF-FTC–treated participants being –1.0% and −3.2%, (Δ = 2.2%; 95% CI, .6%–3.7%; P = .006). The difference between the NRTI components in the mean percentage change in spine BMD was already evident at week 48, at which point the ABC-3TC arms had an estimated mean percentage change of 1.6% (95% CI, .5%–2.8%) smaller than that in the TDF-FTC arms (P = .005).

At week 96, among participants assigned to receive EFV, there was a trend toward a greater decrease in mean percentage change in spine BMD when combined with TDF-FTC than when combined with ABC-3TC (Δ, 1.7%; 95% CI, .04%–3.5%; P = .056). In ATV/r-treated arms, there was a significantly greater decrease in mean percentage change in spine BMD when combined with TDF-FTC than when combined with ABC/3TC (Δ, 2.4%; 95% CI, .4%–4.4%; P = .020, by ITT).

Changes by NNRTI-PI Component: Secondary Analysis. At week 96, by ITT analysis, the mean percentage change in spine BMD was significantly greater in those assigned to ATV/r (−3.1%) than in those in the EFV arm (−1.7%; Δ, −1.5%; 95% CI, −2.8% to −.1%; P = .035). Similar results were seen in the AT analysis. However, at 48 weeks, the mean percentage change was not significantly different between those treated with ATV/r (−3.5%) and those treated with EFV (−2.6%; Δ = −.9%; 95% CI, −2.1% to .2%; P = .11).

Percentage Changes in Hip BMD

The second coprimary analysis involved the mean percentage change in hip BMD at week 96 between the ABC-3TC and the TDF-FTC arms. The estimated mean percentage change in hip BMD for all participants was −3.3% at both weeks 48 and 96 (Table 2). A comparison of ABC-3TC (n = 135) and TDF-FTC (n = 134) with EFV and ATV/r combined was performed, because there was no significant evidence that the treatment effect between these drugs differed at 96 weeks by the NNRTI-PI component (P = .69). Similarly, a comparison of EFV (n = 139) and ATV/r (n = 130) with ABC-3TC and TDF-FTC combined was performed.

Changes by NRTI Components: Primary Analysis. At week 96, ITT analysis showed that the ABC-3TC arms had a significantly smaller decrease in mean percentage change in hip BMD, compared with the TDF-FTC arms (−2.6% vs −4.0%; Δ, 1.4%; 95% CI, .2%–2.5%; P = .024). The AT analysis showed similar results; at week 96, the mean percentage change in hip BMD in the the ABC-3TC arms was −2.6%, compared with −3.9% for TDF-FTC (Δ, = 1.3%; 95% CI, .02%–2.6%; P = .046). The

difference between the NRTI components in the mean percentage change in hip BMD was already evident at week 48, with an estimated mean change of −2.6% for ABC-3TC and −4.1% for TDF-FTC (Δ, 1.5%; 95% CI, .5%–2.5%; $P = .003$).

For persons assigned to receive EFV, at 96 weeks, the mean percentage change in hip BMD was not statistically significantly different between the NRTI components, compared with those assigned to receive ABC-3TC; the estimated mean change was −2.5%, compared with −3.7% for those given TDF-FTC (Δ, 1.2%; 95% CI, −.4% to 2.7%; $P = .15$). There was a trend toward a smaller decrease in mean percentage change in hip BMD for persons given ATV/r with ABC-3TC (−2.7%), compared with those given TDF-FTC (−4.3%; Δ, 1.6%; 95% CI, .2%–3.4%; $P = .075$).

Changes by NNRTI-PI Component: Secondary Analysis. At week 96 and by ITT analysis, the mean percnetage change in hip BMD was not statistically significantly different between EFV and ATV/r (Δ, −.3%; 95% CI, −1.5% to .9%; $P = .61$). Similar results were seen in the AT analysis and at week 48.

Changes in Spine and Hip BMD Adjusted for Baseline Covariates

The ITT analyses of mean percentage change from entry to week 96 of spine and hip BMD were adjusted for the following prespecified baseline covariates that could affect BMD, first individually and then jointly, with use of linear regression: NNRTI-PI (or NRTI components for the NNRTI-PI analyses), spine BMD (or hip BMD for corresponding analysis), sex, age, race/ethnicity, $\log_{10}$ HIV-1 RNA load, CD4 cell count, and BMI. For analyses of the NRTI component effect or the NNRTI-PI component effect, all of the adjusted models led to results similar to those of the unadjusted analyses.

Association Between Baseline Factors and Changes in BMD at 96 Weeks

Table 3 summarizes the linear regression analyses that were performed to assess the baseline factors associated with 96-week percentage change in spine and hip BMD. The covariates included in the model were the same as the ones mentioned in the previous paragraph. For spine BMD, in addition to the significant ABC-3TC and ATV/r effects, in both univariate and multivariable models, higher baseline CD4 cell count was independently associated with significant increases, and higher baseline $\log^{10}$ HIV-1 RNA load was independently associated with significant decreases in spine BMD at 96 weeks. For hip BMD, in addition to the significant ABC-3TC effect, in univariate and multivariable models, higher baseline BMI was independently associated with significant increases at 96 weeks.

Timing of BMD Changes: Repeated Measures Analyses. To understand the dynamics of BMD change over time, an analysis of the slopes of changes in the early phase (0–48 weeks) and late phase (48–192 weeks) was explored in and between study components. For spine BMD, as shown in Table 4, there was a statistically significant difference between the NRTIs in the

Table 3. Results of the Regression Analysis

Variable	No. of participants	Parameter estimate	95% CI	*P* value[a]
96-week percentage change in lumbar spine BMD, univariate analyses[b]				
ABC-3TC (vs TDF-FTC)	198	2.00	(.66–3.33)	.004
ATV/r (vs EFV)	198	-1.46	(-2.82–.10)	.035
Baseline HIV-1 RNA (per $\log_{10}$ copies/mL)	198	-2.00	(-3.00–1.01)	<.001
Baseline CD4 cell count (per 50 cells/μL)	198	0.48	(.28–.68)	<.001
96-week percentage change in lumbar spine BMD, multivariable analysis[c]				
ABC-3TC (vs TDF-FTC)	198	1.90	(.64–3.17)	.003
ATV/r (vs EFV)	198	-1.38	(-2.70–.07)	.039
Baseline HIV-1 RNA (per $\log_{10}$ copies/mL)	198	-1.17	(-2.30–.05)	.041
Baseline CD4 cell count (per 50 cells/μL)	198	0.37	(.14–.59)	.001
96-week percentage change in hip BMD, univariate analyses[b]				
ABC-3TC (vs TDF-FTC)	195	1.35	(.18–2.53)	.024
Baseline BMI (per kg/m^2)	195	0.16	(.02–.29)	.021
96-week percentage change in hip BMD, multivariable analysis[c]				
ABC-3TC (vs TDF-FTC)	195	1.28	(.10–2.46)	.033
Baseline BMI (per kg/m^2)	195	0.18	(.04–.32)	.013

NOTE. [a] Only *P* values < .050 are presented.

[b] Individually assessed sex, age, race/ethnicity, $\log_{10}$ HIV-1 RNA, CD4 count, BMI, ABC-3TC, and ATV/r.

[c] Jointly assessed sex, age, race/ethnicity, $\log_{10}$ HIV-1 RNA load, CD4 cell count, BMI, ABC-3TC, and ATV/r.

Table 4. Repeated Measures Analysis, BMD (Percentage Change)

BMD site	Time interval		Mean percentage change/year	*P* value	95% CI
Lumbar spine	Entry to week 48	TDF/FTC	−4.09	<.001	(−4.87–3.30)
		ABC/3TC	−2.43	<.001	(−3.25–1.60)
		Difference (ABC/3TC - TDF/FTC)	1.66	.005	(.52–2.80)
	Week 48 to week 192	TDF/FTC	0.00	1.00	(−.34–0.34)
		ABC/3TC	0.60	.002	(.22–.98)
		Difference (ABC/3TC - TDF/FTC)	0.60	.022	(.09–1.11)
	Entry to week 48	EFV	−2.77	<.001	(−3.58–1.95)
		ATV/r	−3.70	<.001	(−4.52–2.88)
		Difference (ATV/r - EFV)	−0.93	.11	(−2.09–.22)
	Week 48 to week 192	EFV	0.44	.016	(.08–.79)
		ATV/r	0.12	.51	(−.24–.49)
		Difference (ATV/r - EFV)	−0.31	.23	(−.83–.20)
Hip	Entry to week 48	TDF/FTC	−4.29	<.001	(−5.02–3.56)
		ABC/3TC	−2.86	<.001	(−3.60–2.11)
		Difference (ABC/3TC - TDF/FTC)	1.43	.007	(.39–2.47)
	Week 48 to week 192	TDF/FTC	0.27	.14	(−.09–.62)
		ABC/3TC	0.41	.017	(.07–.74)
		Difference (ABC/3TC - TDF/FTC)	0.14	.56	(−.34–.63)
	Entry to week 48	EFV	−3.38	<.001	(−4.19–2.57)
		ATV/r	−3.74	<.001	(−4.42–3.06)
		Difference (ATV/r - EFV)	−0.36	.50	(−1.42–.69)
	Week 48 to week 192	EFV	0.30	.065	(−.02–.62)
		ATV/r	0.36	.061	(−.02–.73)
		Difference (ATV/r - EFV)	0.06	.82	(−.44–.55)

slope of BMD change during both the early and the late phase, favoring ABC-3TC. Of interest, ABC-3TC arms, but not TDF-FTC, had a significant positive spine BMD percentage change per year during the late phase. For NNRTI-PI components, there was no statistically significant difference in the slopes between NNRTI and PI arms in either phase, with both arms having decreasing BMD during the early phase and only EFV significantly increasing spine BMD in the late phase.

For hip BMD, the treatment differences and kinetics of bone loss were similar, with most of the BMD loss occurring during the first 48 weeks in both NRTI arms. During the late phase, ABC-3TC arms again showed a significant gain in hip BMD. Both EFV and ATV/r arms lost bone in the first phase, but the slope of the late phase did not reach statistical significance in either arms.

On-Study Bone Fractures

On-study bone fractures were collected in A5224s and in the A5202 parent study ($n = 1857$). In the substudy, 15 participants (5.6%) reported a bone fracture, all of which were a result of trauma, with 10 occurring in the EFV arms. There were no statistically significant differences in the number of fractures between the NRTIs ($P = 1.00$) or the NNRTI and PI study arms ($P = .29$). Similarly, there was no statistically significant difference in time to first bone fracture between NRTI ($P = .76$) or NNRTI/PI study arms ($P = .27$).

In the parent study-A5202, 80 participants (4.3%) reported at least one bone fracture on study (ABC-3TC plus EFV, 4.7%; ABC-3TC plus ATV/r, 3.5%; TDF-FTC plus EFV, 4.5%; and TDF-FTC plus ATV/r, 4.5%). Among these, 10 (12.7%) were atraumatic. The bone fractures were balanced across the study arms, with no statistically significant differences between the NRTI ($P = .73$) or the NNRTI and PI components ($P = .57$). No statistically significant difference in time to first bone fracture was seen between the NRTIs ($P = .71$) or the NNRTI and PI components ($P = .49$). Similarly, incidence rates were similar across arms (ABC-3TC plus EFV, 1.9 cases per 100 patient-years; ABC-3TC plus ATV/r, 1.4 cases per 100 patient-years; TDF-FTC plus EFV, 1.8 cases per 100 patient-years; and TDF-FTC plus ATV/r, 1.8 cases per 100 patient-years).

DISCUSSION

This report details changes in BMD in participants randomized to receive 1 of 4 frequently used regimens for treatment of HIV infection. As shown in prior studies, we demonstrated that ART initiation led to a large initial decrease in BMD, with ABC-3TC plus EFV being the only regimen studied that did not lead to a statistically significant decrease in spine BMD at week 96. We also found that TDF-FTC led to greater decreases in spine and hip BMD than did ABC-3TC and that ATV/r induced a significantly greater decrease in the spine BMD than did EFV. Our results are robust, because correcting for potential confounders and/or imbalances, including traditional bone risk factors and HIV disease characteristics, did not affect these results. AT analyses yielded results similar to those of ITT analyses. The incidence of fractures did not differ significantly between the regimen components.

The present study adds to a body of literature demonstrating a greater effect on reducing BMD with TDF-based therapies, compared with other regimens [11, 15]. A previous randomized clinical trial involving ART-naive participants compared TDF-FTC with ABC-3TC, both with EFV [11]. At 48 weeks, decreases in spine and hip BMD were significantly greater with TDF-FTC. Our data are consistent with these results and extend the observation to 96 weeks and to the use of both EFV and ATV/r.

The role of PI therapy in HIV-associated osteoporosis has been debated. Our study revealed a greater decrease in BMD with ATV/r regimens, compared with EFV, but only at the spine. A trend toward greater decrease in total body BMD with another ritonavir-boosted PI (lopinavir/r), compared with EFV, was observed in another randomized trial [16]. By contrast, other studies have not shown an effect of PI on BMD [17, 18]. Some of these discrepancies may be related to the use of whole-body DXA instead of using the more sensitive site-specific bone DXAs. Our study showed that the effect of ART varies by site, supporting the use of site-specific DXA. This site differential effect could be attributable to the trabecular nature of vertebral bone, which is more active and more subject to bone turnover and remodeling, compared with cortical (eg, hip) bone. In addition, different PIs may have differential effects on bone, analogous to their variable effect in the drug class on lipid changes.

The mechanisms involved in bone loss after initiation of ART are not well understood. TDF may affect bone through proximal tubule toxicity, resulting in phosphate wasting and increased bone turnover [19]. EFV and PIs may affect BMD indirectly through vitamin D metabolism [20–26]. In multivariable analysis exploring the factors associated with BMD changes at 96 weeks, we found that, in addition to TDF-FTC and ATV/r each leading to greater spine BMD decrease, compared with ABC-3TC and EFV, respectively (also in hip BMD for TDF-FTC), other baseline factors were associated with BMD loss. Some of these (eg, lower BMI) are also associated with BMD decreases in the general population. Relevant HIV-specific factors that decreased BMD include higher baseline HIV-1 RNA load and lower CD4 cell count, corroborating findings of other studies showing a greater risk of osteopenia and/or osteoporosis in those with longer HIV infection duration [27–29]. These observations support the fact that HIV infection or immunologic factors linked to HIV infection play a role in bone loss after treatment initiation. Indeed, HIV proteins can increase osteoclastic activity [30] and promote osteoblast apoptosis [31, 32]. Furthermore, cytokines, such as IL-6 and TNF-α, may stimulate osteoclast activity [33–35].

Because most of our study participants were young (median age, 38 years), with a relatively low risk of falls, it was not surprising that we did not observe an increased rate of fractures with specific ART regimens. However, the degree of BMD loss and the between-component differences should not be perceived as clinically insignificant. Indeed, these decreases are similar in magnitude to the BMD losses sustained during the first 2 years of menopause [36]. Furthermore, our study population was young and mostly (85%) male, a group typically spared significant loss of BMD. In the general population, the mean 2-year change in BMD in men 20–49 years of age is −0.8% at the hip and −0.3% at the lumbar spine [37]. Even at the most vulnerable skeletal time in women (during the first 2 years of menopause), the loss of BMD accelerates, with mean annual rates of bone loss of 1.2% –1.6%. This magnitude of bone loss is equivalent to the point estimates of the mean differences shown between the ABC-3TC and TDF-FTC regimens at the hip and spine and between EFV and ATV/r at the spine, although the confidence intervals are consistent with smaller differences in the means.

Our study is notable for the observations regarding timing of BMD changes after ART initiation. Large early reductions in spine and hip BMD were observed within the first 48 weeks after ART initiation with all regimens. After the initial 48 weeks (cutoff chosen by inspection), BMD did not change or even improved slightly with some of the regimens. This is consistent with prior ART initiation studies and with longitudinal studies of treatment-experienced participants that have shown stability in BMD over time [3, 28, 38, 39].

Our study has several limitations. First, the duration of follow-up for study of bone end points was relatively short. Nevertheless, to our knowledge, our study has the longest follow-up of published prospective longitudinal studies of BMD after ART initiation. Second, the changes in the NRTI backbone of the regimen that resulted from the outcome of the DSMB review of A5202 were relatively frequent. However, our ITT results were consistent with our AT results. Other limitations are that the NNRTI-PI component was provided in an open-labeled fashion and that there was a high amount of missing data, which is not unusual for large multicentered studies. Finally, the study did not collect smoking and alcohol status, which could affect BMD.

In conclusion, we revealed that the initiation of ART leads to prompt reductions in spine and hip BMD observed within the

first 48 weeks, independent of ART type. At week 96, TDF-FTC, both in the spine and hip, and ATV/r in the spine produced significantly more bone loss than did ABC-3TC– or EFV-based regimens. Studies investigating the mechanisms behind the bone loss with ART initiation are needed.

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