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# Tenofovir Disoproxil Fumarate and an Optimized Background Regimen of Antiretroviral Agents as Salvage Therapy: Impact on Bone Mineral Density in HIV-Infected Children

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## ABSTRACT

**OBJECTIVE.** Tenofovir disoproxil fumarate, a nucleotide analog HIV reverse transcriptase inhibitor with demonstrated activity against nucleoside-resistant HIV, is approved for use in adults but not children. Metabolic bone abnormalities have been seen in young animals given high-dose tenofovir and HIV-infected adults that were treated with oral tenofovir disoproxil fumarate. However, tenofovir disoproxil fumarate is being used in children despite a lack of bone safety data. We hypothesized that, given the higher rate of bone turnover that is associated with normal skeletal growth, the potential for TDF-related bone toxicity may be greater in children than in adults.

**METHODS.** Fifteen highly antiretroviral-experienced HIV-infected children who were 8 to 16 years of age (mean  $\pm$  SD: 12  $\pm$  2) and required a change in therapy received tenofovir disoproxil fumarate 175 to 300 mg/m<sup>2</sup> per day (adult dose equivalent) as part of highly active antiretroviral therapy for up to 96 weeks. Bone mineral density of the lumbar spine, femoral neck, and total hip by dual-energy x-ray absorptiometry and blood and urine markers of bone metabolism were measured at 0, 24, 48, 72, and 96 weeks.

**RESULTS.** Median *z* score (SD score compared with age, gender, and ethnicity-matched control subjects) of the lumbar spine, femoral neck, and total hip were decreased from baseline at 24 weeks and 48 weeks and then stabilized. Lumbar spine bone mineral apparent density (which estimates volumetric bone mineral density independent of bone size) *z* scores also decreased at 24 weeks. Absolute decreases in bone mineral density were observed in 6 children; the mean age of these children was significantly younger than the bone mineral density stable group (10.2  $\pm$  1.1 vs 13.2  $\pm$  1.8 years). The change in lumbar spine bone mineral

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### Key Words

bone mineral density, HIV, bone mineralization, antiretroviral therapies

### Abbreviations

BMD—bone mineral density  
HAART—highly active antiretroviral therapy  
TDF—tenofovir disoproxil fumarate  
LS—lumbar spine  
PTH—parathyroid hormone  
FN—femoral neck  
TH—total hip  
DXA—dual-energy x-ray absorptiometry  
BMAD—bone mineral apparent density

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density correlated with decreases in HIV plasma RNA during treatment. Metabolic markers of bone formation and resorption were variable. Two children in whom tenofovir disoproxil fumarate was discontinued because of bone loss that exceeded protocol allowances demonstrated partial or complete recovery of bone mineral density by 96 weeks.

**CONCLUSIONS.** Tenofovir disoproxil fumarate use in children seems to be associated with decreases in bone mineral density that, in some children, stabilize after 24 weeks. Increases in bone markers and calcium excretion suggest that tenofovir disoproxil fumarate may stimulate bone resorption. Bone turnover is higher in children than in older adolescents and adults because of skeletal growth, potentially explaining the greater effect seen in young children. Decreases in bone mineral density correlate with decreases in viral load and young age, suggesting that young responders may be at greater risk for bone toxicity.

**H**IV INFECTION IS a known risk factor for altered bone mineral metabolism in children.<sup>1</sup> In addition to HIV-associated abnormalities in growth factors and cytokines, which may be responsible for decreased bone mineral density (BMD),<sup>2,3</sup> the use of highly active antiretroviral therapy (HAART) seems to contribute to bone loss, possibly by increasing bone turnover.<sup>4,5</sup>

Tenofovir disoproxil fumarate (TDF), an orally available prodrug of the nucleotide reverse transcriptase inhibitor tenofovir, is approved for use as part of combination therapy for HIV-infected adults. An important safety concern for TDF use in adults is the potential for

adverse effects on bone metabolism, demonstrated in both laboratory animals<sup>6–9</sup> and humans.<sup>10</sup> Because of its unique resistance profile and the critical need for new non-cross-resistant HAART regimens in pediatrics, TDF currently is used off-label in HIV-infected children who are unresponsive to more traditional HAART. We hypothesized that, given the higher rate of bone turnover that is associated with normal skeletal growth, the potential for TDF-related bone toxicity may be greater in children than in adults. To test this hypothesis, we studied the bone effects of TDF in children with highly drug-resistant HIV treated with TDF-containing salvage regimens as part of a phase 1b/2a study of TDF.<sup>11,12</sup>

## METHODS

### Patients

Nineteen highly antiretroviral-experienced HIV-infected children who required a change in therapy were enrolled in single-center, single-arm, open-label, phase 1b/2a trial to evaluate TDF treatment (175–300 mg/m<sup>2</sup> per day; median dose: 208 mg/m<sup>2</sup> per day; range: 161–256 mg/m<sup>2</sup> per day), in addition to other HAART. At entry into the study, patients began an optimized background regimen of antiretrovirals 6 days after initiation of TDF (Table 1). Inclusion criteria were age >4 and <18 years, body surface area ≥0.50 m<sup>2</sup>, plasma HIV RNA levels ≥10 000 copies/mL, history of failing at least 2 previous antiretroviral regimens, and ability to swallow tablets. Exclusion criteria were concurrent treatment with nephrotoxic agents, cancer chemotherapy, or other investigational agents. The original protocol called for discontinuation of TDF for a confirmed decrease in lumbar spine (LS) BMD >6%. After the first patient expe-

**TABLE 1** Baseline Data for 15 Patients Included in Analysis

Patient	Age, y	Gender	Height SDS	Weight SDS	BMI SDS	Puberty, Tanner Stage <sup>a</sup>	Time on TDF, wk	Other Medications <sup>b</sup>
1	10	M	-1.6	-0.7	0.4	1	96	ZDV, 3TC, LPV/RTV/SQV, fluticasone
2	11	M	1.1	1.4	1.4	3	96	d4T, EFV, LPV/RTV, fluticasone
3	16	F	0.2	1.7	1.7	4	96	ddl, EFV, LPV/RTV
4	14	M	-3.1	-3.1	-1.5	1	36	ZDV, 3TC, APV, RTV
5	14	F	-1.6	-1.0	-2.4	3	96	d4T, 3TC, LPV/RTV, fluticasone
6	10	M	-0.5	-0.8	-0.7	1	96	ZDV, 3TC, LPV/RTV, topical hydrocortisone
7	11	M	-2.4	-2.5	-1.4	1	32	ZDV, 3TC, LPV/RTV
8	8	M	-1.3	-0.9	-0.2	1	50	ddl, EFV, LPV/RTV, triamcinolone
9	13	F	-2.4	-1.9	-0.8	2	96	ZDV, 3TC, EFV, LPV/RTV, fluticasone/salmeterol, topical hydrocortisone
11	15	F	1.2	0.7	0.3	4	96	ddl, LPV/RTV
12	14	M	-0.6	-0.8	-0.8	2	60	d4T, 3TC, IDV, RTV
15	11	M	0	0.9	1.32	1	48	ZDV, 3TC, ABC, LPV/RTV, fluticasone
16	12	F	0.4	0.7	0.8	3	96	ZDV, 3TC, ABC, LPV/RTV, fluticasone
17	10	M	-0.1	-0.9	-1.33	1	48	d4T, 3TC, LPV/RTV, SQV, fluticasone
18	11	M	-2.8	-2.9	-1.35	1	96	d4T, ABC, LPV/RTV, topical hydrocortisone

Some patient numbers are missing, reflecting the 4 excluded patients. M indicates male; F, female; SDS indicates SD score; ZDV, zidovudine; 3TC, lamivudine; LPV, lopinavir; RTV, ritonavir; SQV, saquinavir; d4T, stavudine; EFV, efavirenz; ddl, didanosine; ABC, abacavir; IDV, indinavir; APV, amprenavir.

<sup>a</sup> Tanner stage was determined by visual inspection of breasts in girls and pubic hair in boys.

<sup>b</sup> Other medications include antiretrovirals and steroid preparations. No steroids therapies were systemic; all were topical, inhaled, or intranasal. Inhaled fluticasone doses ranged from 100 to 200 µg/day.

rienced such a decrease in the setting of virologic and immunologic benefit, the protocol was amended so that patients experiencing >6% decreases in LS BMD could continue being treated with TDF provided they had not experienced fragility fractures, had BMD z scores greater than -2.5, and had experienced at least a stabilization in viral load or CD4 count ( $\geq 0.5 \log_{10}$  decrease in HIV plasma RNA or  $\geq 25\%$  absolute CD4 count increase). Patients who did not meet these conditions discontinued TDF but remained on study for follow-up. The National Cancer Institute Institutional Review Board approved the protocol. Informed consent was obtained from the parents or guardians, and minors' assent was obtained from the patients.

### Clinical Assessments

Clinical assessments were performed at baseline and then at 4- to 12-week intervals. Height was measured using a wall-mounted stadiometer, and pubertal staging was assessed by visual inspection according to the method of Tanner.<sup>13,14</sup> Height, weight, and BMI z scores (SD score compared with age- and gender-matched control subjects) were calculated using the 2000 Centers for Disease Control and Prevention database.<sup>15</sup>

### Laboratory Assessments

Metabolic markers of bone metabolism were measured at baseline and at 24, 48, 72, and 96 weeks. Serum was assayed for calcium, phosphorus, intact parathyroid hormone (PTH; immunochemiluminometric assay), 25-OH vitamin D (immunochemiluminometric assay), and 2 markers of osteoblastic bone formation: bone-specific alkaline phosphatase (immunoenzymatic assay) and morning osteocalcin (immunochemiluminometric assay). Twenty-four-hour urine collections were assessed for calcium, creatinine, and phosphorus excretion and 2 markers of osteoclastic bone resorption: collagen cross-linked N-telopeptides (competitive immunoassay) and deoxypyridinoline (high-pressure liquid chromatography). Plasma HIV RNA levels (Roche Amplicor Monitor 1.5; Roche Diagnostics, Alameda, CA), enumeration of lymphocytes and lymphocyte subsets, and routine laboratory monitoring also were performed at every visit and were reported previously.<sup>11</sup>

### Radiologic Assessments

BMD of the postero-anterior LS, femoral neck (FN), and total hip (TH) were assessed by dual-energy x-ray absorptiometry (DXA; QDR 4500; Hologic, Waltham, MA) at baseline and at 24, 48, 72, and 96 weeks using standard scanning techniques. In patients who experienced a >6% loss of BMD, an additional confirmatory scan was performed 3 months after the scan in question. Scans were obtained in the array mode and analyzed using medium- or low-density software (versions 12.1 and 11.2; Hologic), where appropriate. All scans were

reviewed by 2 nonblinded investigators (R.I.G. and J.C.R.) for adequacy of bone map and comparability of bone area with previous scans. To decrease possible bias by the investigators, previous bone mineral content, BMD, and z score measurements were not used to assess adequacy of the scans. In our institution, the precision of DXA in adults is 1.1% at the LS, 1.8% at the FN, and 0.9% at the TH. In children who are 9 years or older, z scores were calculated using the gender-, age-, and ethnicity-specific pediatric normative database published by Bachrach et al.<sup>16</sup> In the 1 patient who was 8 years at entry into the study, the gender- and age-specific normative database by Faulkner et al.<sup>17</sup> was used. Bone mineral apparent density (BMAD) of the LS, a calculation from the 2-dimensional DXA based on geometric assumptions of the vertebral body, estimates volumetric bone density and therefore largely is independent of bone size.<sup>18</sup> BMAD was determined as follows: LS bone mineral content/(LS area)<sup>1.5</sup>. Z scores were calculated.<sup>16</sup> Low BMD was defined as a z score of less than -2. In children with open growth plates, radiographs of the left hand and wrist were obtained every 6 months and evaluated by a single nonblinded observer (R.I.G.).

### Statistical Analysis

Results are expressed as the median (range) for all data points that were available at each time point unless otherwise indicated. Comparisons between time points were made using a Wilcoxon signed rank test. Age comparisons were made using a *t* test. Relationships between specific independent variables and the change in LS BMD were determined using standard linear regression. All *P* values are 2-tailed and were not adjusted for multiple comparisons.

### RESULTS

Individual patient characteristics and concurrent medications are listed in Table 1. Other than HAART, no patients were treated with long-term systemic medications that are known to affect bone mineral acquisition, such as glucocorticoids. All patients were prescribed a daily multivitamin that contained 400 IU of vitamin D and 40 to 100 mg of elemental calcium; however, compliance with this medication was not monitored. Four patients discontinued treatment within 18 weeks of starting TDF and were excluded from analysis; 1 patient never received TDF because of baseline elevated transaminases, and 3 discontinued because of transaminase elevation while taking TDF. Six patients discontinued TDF after 24 weeks; their results are included in the grouped data up to and including the last bone density, metabolic, and clinical evaluation before drug stoppage. Two of these patients (patients 8 and 17) discontinued because of significant decreases in bone density, 3 (patients 4, 12, and 15) discontinued for reasons unrelated to bone toxicity, and 1 patient (patient 7) died of HIV.

One patient (patient 4) was restricted to a wheelchair because of cerebral palsy of the lower extremities; only BMD data from his LS were included in the analysis. One patient (patient 18) did not have a FN and TH DXA performed at baseline; therefore, no comparisons between time points were made. No fractures were sustained by any of the patients during the study. The pharmacokinetic and clinical responses and the non-bone-related toxicities of these patients were described previously.<sup>11,12</sup>

### Changes in Bone Density

Median (range) *z* scores at baseline were as follows: LS, -1.2 (-3.0 to 1.9); TH, -1.0 (-2.9 to 2.9); FN, -1.4 (-3.6 to 3.8); and LS BMAD, -0.9 (-3.2 to 1.2). Six of the patients (patients 6, 7, 9, 11, 17, and 18) had low *z* scores (less than -2) at baseline for at least 1 of the sites measured. Median *z* scores of the LS, TH, and FN were decreased compared with baseline at 24 and 48 weeks (Fig 1). These decreases seemed to stabilize after this time point; however, the differences at 72 and 96 weeks were not statistically different from baseline, likely as a consequence of the reduced number of patients at these time points and exclusion of the 2 patients with markedly decreased BMD. Similarly, LS BMAD *z* scores were significantly decreased at 24 weeks (Fig 1). The decreases in BMD *z* score were not associated with decreases in height *z* score, which remained stable throughout the study (-0.6 [-3.1 to 1.2] at baseline vs -0.1 [-3.0 to 1.5] at 96 weeks; *P* = .43). The decreases in BMD also were not attributable to weight loss because the median weight *z* score did not change significantly during the study (-0.8 [-3.1 to 1.7] at baseline vs -1 [-3.5 to 1.7] at 96 weeks; *P* = .06). These BMD changes reflect decreases (>1%) in measured LS BMD that were observed in 6 of the 15 patients (Fig 2). None of these 6 patients had a low LS BMD *z* score (less than -2) at baseline. The

mean age of the group with decreasing BMD was significantly younger than the BMD stable group ( $10.2 \pm 1.1$  vs  $13.2 \pm 1.8$  years; *P* = .003); however, the correlation between BMD decreases and age was only weak to moderately strong (Fig 2). Decreases in BMD at 24 weeks were negatively correlated with decreases in HIV plasma viral load (Fig 3).

### Biochemical Changes

Mean serum levels of calcium, phosphorus, and 25-OH-vitamin D were normal throughout the study and did not change significantly with treatment (data not shown). Baseline levels of 25-OH-vitamin D were low (<20 ng/mL) in 2 patients; the vitamin D level normalized in 1 patient (patient 5) by 48 weeks. The other patient's (patient 2) vitamin D level remained low throughout the study; this individual was 1 of the 6 who experienced an initial drop in BMD, but his BMD returned to baseline by 48 weeks and subsequently continued to increase. Intact PTH had decreased significantly at 48 weeks (31 pg/mL [11–76 pg/mL] at baseline vs 21 pg/mL [15–62 pg/mL] at 48 weeks; *P* = .048); although the median PTH also was lower at 96 weeks, the difference was not statistically significant (27 pg/mL [14–43 pg/mL]; *P* = .25).

Twenty-four-hour urine calcium excretion was increased at 24 weeks compared with baseline (3.5 mg/kg per day [0.83–11.7 mg/kg per day] vs 2.3 mg/kg per day [0–9.3 mg/kg per day]; *P* = .02) but had returned to baseline by 48 weeks. One patient developed nephrolithiasis at week 26 that required hospitalization and temporary discontinuation of TDF. Tubular resorption of phosphorus seemed to decline, and although this decrease was significant at 24 weeks (95% [87%–99%] vs 92% [86%–99%]; *P* = .02), the change was not statistically significant on subsequent measurements. These returns to baseline may reflect the high dropout rate,

**FIGURE 1**  
BMD *z* scores of the LS, FN, and TH and BMAD *z* scores of the LS during TDF treatment. \**P* < .05 compared with baseline (time 0). Upper and lower borders of the box represent 75th and 25th percentiles, respectively. Median is indicated by horizontal line within the box. ●, Outlier values.

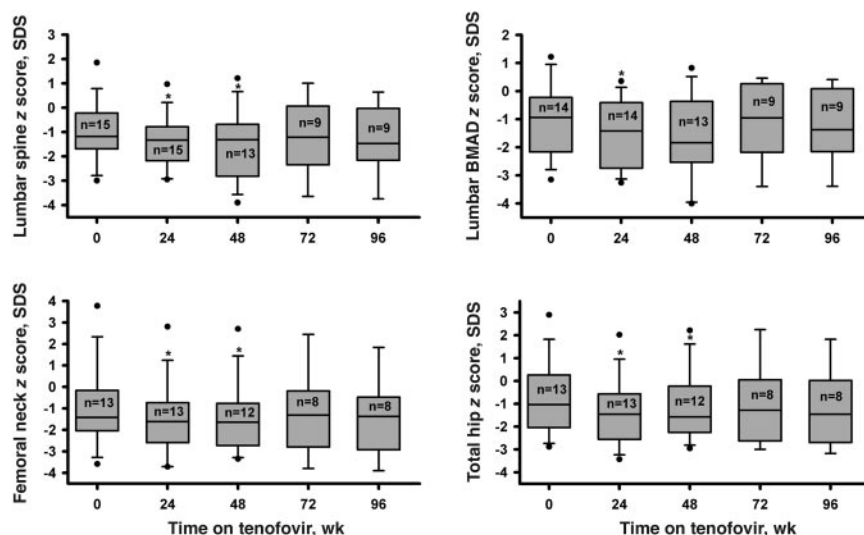


FIGURE 2

Left, Percentage change in absolute LS BMD during TDF for individual patients. Only patients who showed a decrease in BMD at 24 weeks are labeled by patient number, as shown by the filled symbols. Right, Percentage change in absolute LS BMD from baseline after 24 weeks TDF treatment for age ( $r^2 = 0.215$ ;  $P = .08$ ).

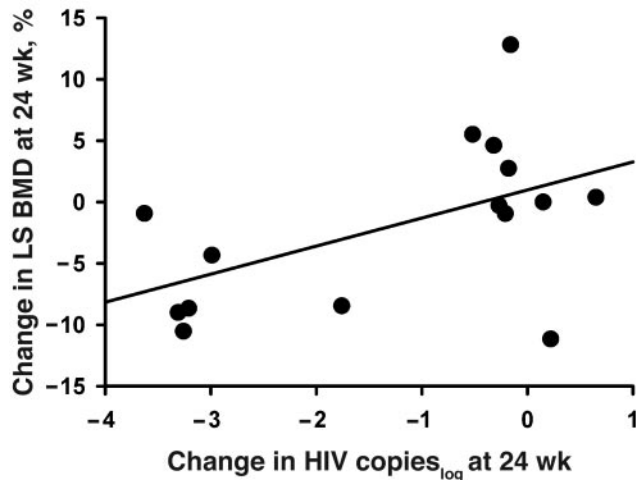
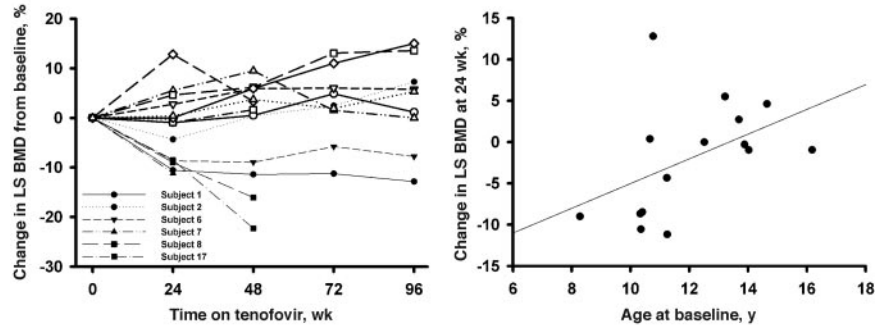


FIGURE 3

Percentage change in LS BMD versus HIV RNA copies<sub>log</sub> at 24 weeks. ( $r^2 = 0.275$ ;  $P < .05$ ).

rather than true clinical improvement. Similarly, serum osteocalcin was increased compared with baseline throughout the study; however, the differences were significant only at 24 and 48 weeks. Urinary collagen cross-linked N-telopeptides and serum bone-specific alkaline phosphatase tended to increase, but these changes were not statistically significant (data not shown).

#### Recovery of BMD After TDF Discontinuation

Two patients discontinued TDF after 48 to 50 weeks because of significant decrease in BMD. In patient 8, these decreases were seen in all 3 sites assessed and were associated with a marked increase in urine calcium excretion. By 96 weeks, absolute BMD had returned to baseline (Fig 4); however, z scores remained lower given the patient's increased age. Similarly, in patient 17, LS BMD showed near recovery by 96 weeks (Fig 4); however, TH and FN BMD continued to decline even after discontinuation of TDF. Both patients remained on their other antiretrovirals throughout the study; saquinavir was initiated in patient 17 after TDF was discontinued. HIV disease remained stable after discontinuation of TDF in both patients.

#### Hand Radiographs

Twelve patients had hand radiographs performed at baseline; 3 patients had fused epiphyses. In 4 of the 9 patients with unfused epiphyses, the zone of provisional calcification (area of newly forming trabecular bone located just beneath the growth plate) appeared decreased in thickness at 24 weeks compared with baseline (Fig 5). Three of the 4 patients with this finding also had decreases in BMD during the study.

#### DISCUSSION

TDF treatment in young, highly antiretroviral-experienced HIV-infected children resulted in a significant decrease in absolute BMD and BMD z score, with an increase in urinary calcium excretion. Decreases in BMD were notable in younger growing children demonstrating a good virologic response to TDF. These changes seemed to stabilize by 24 weeks; however, in 2 patients with continuing bone loss, stabilization and recovery did not occur until after discontinuation of TDF. Biochemical markers of bone metabolism tended to increase but were not statistically significant, likely as a result of the small number of patients and high dropout rate. Despite a history of severe vertically acquired HIV disease, only one third of our patients had decreased BMD at baseline, and no patients experienced fractures during the study period.

Many studies have documented bone loss in HAART-treated HIV-infected adults, particularly those who received protease inhibitors.<sup>19–21</sup> Studies also have reported decreased BMD in HIV-infected children, especially those who were treated with HAART.<sup>1,4,5</sup> A study that compared TDF with stavudine in antiretroviral-naïve adults demonstrated a greater decline in spinal BMD at 24 and 48 weeks in the TDF-treated group that, similar to our findings, seemed to stabilize but not recover thereafter.<sup>10</sup> The incidence of bone fractures was no different between groups. The magnitude of spinal bone loss was lower in the adults (2%–3%),<sup>10</sup> compared with the >6% loss in spinal BMD that was seen in 5 of our young patients. This difference likely is attributable to the lower rates of bone turnover in older adolescents and adults, who have completed their skeletal growth.

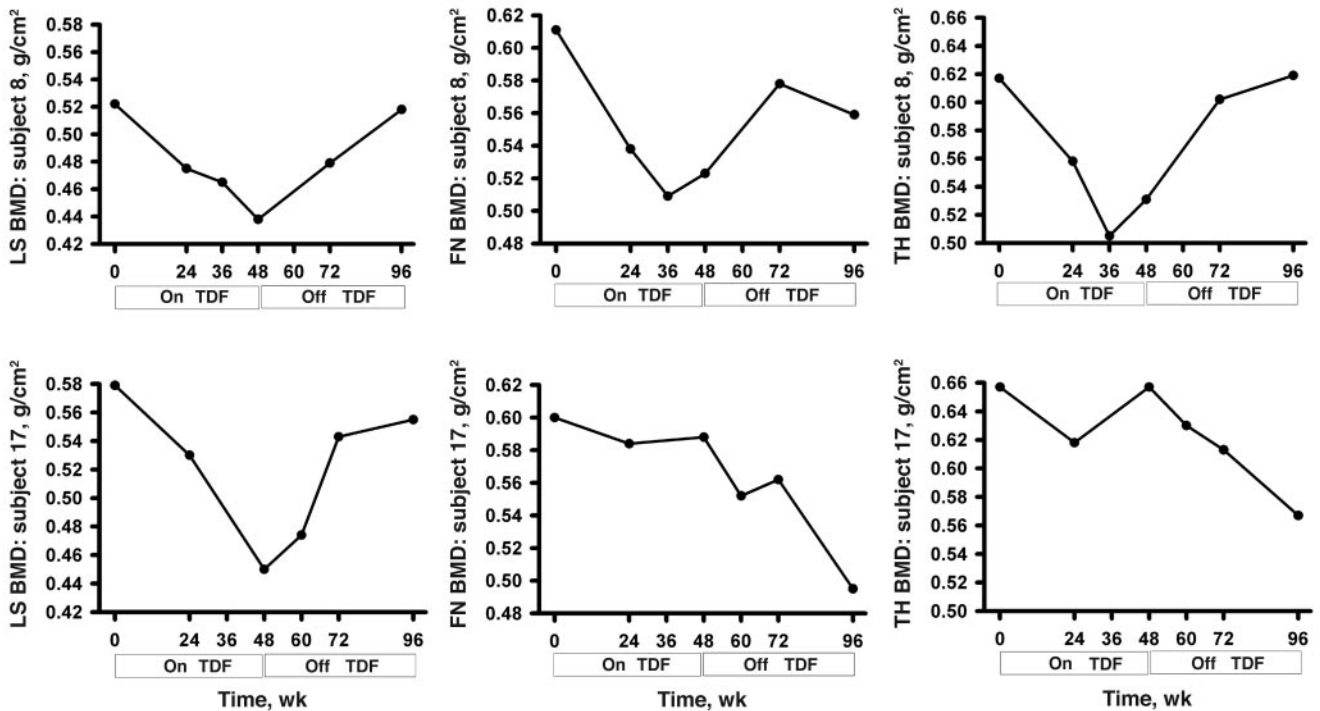


FIGURE 4  
BMD ( $\text{g}/\text{cm}^2$ ) of the LS, FN, and TH in the 2 patients who discontinued TDF at 48 to 50 weeks because of bone loss.

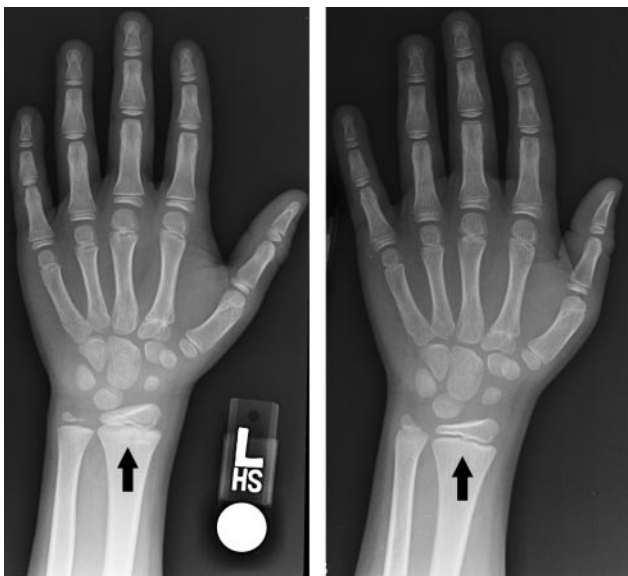


FIGURE 5  
Hand/wrist radiograph of patient 1 at baseline (left) and after 24 weeks of TDF (right). Cortices appear thinner at 24 weeks, and there is decreased new trabecular bone in the areas under the growth plate (primary spongiosa, indicated by arrows).

A longitudinal study in children treated with non-TDF-containing HAART demonstrated both a normal rate of LS BMD increase and a decreased rate of total body bone mineral acquisition compared with control subjects.<sup>4</sup> A recent study by the same group evaluated the effect of replacing stavudine with TDF on bone min-

eral accrual in children aged 6 to 18 years.<sup>22</sup> In contrast to our study, the patients did not experience absolute bone loss after switching to TDF, and the authors did not detect a difference in the rate of bone mineral accrual while the patients were on TDF, which was similar to healthy control subjects. The reasons for our different findings are not certain but likely are related to the difference in patient population. The patients in the study by Giacomet et al<sup>22</sup> were older and healthier and had greater height and weight z scores than our patients. More important, the majority of their patients were in middle to late puberty or postpubertal, whereas most of our patients were prepubertal or in early puberty. As our data suggest, TDF-related bone loss may be greater in less skeletally mature children. In addition, because the patients in our study received protease inhibitors as part of their combination therapy, unlike the patients in the study by Giacomet et al, who all received tenofovir/lamivudine/efavirenz combination therapy, the tenofovir concentrations that were experienced by our patients may have been higher.<sup>11,12</sup> Most pediatric patients who receive tenofovir as part of a salvage antiretroviral therapeutic regimen also will receive a protease inhibitor, so toxicities that are observed in the context of protease-inhibitor combination therapy may be more likely to reflect the clinical experience of pediatric salvage patients who are treated with tenofovir.

High-dose tenofovir treatment has been shown to cause skeletal abnormalities in juvenile animals. At

doses of 30 mg/kg per day for >8 months, tenofovir treatment in newborn and infant rhesus macaques was associated a rachitic-type picture, including widened growth plates, bony deformities, growth restriction, increased alkaline phosphatase, and hypophosphatemia; these changes were not seen in animals that were given tenofovir in lower doses or for shorter durations.<sup>9</sup> Similarly, histomorphometric studies in growing simian immunodeficiency virus–infected rhesus monkeys demonstrated that high-dose tenofovir induced a mineralization defect in newly forming cortical bone and widened osteoid seams, as seen in rickets/osteomalacia.<sup>6</sup> Insulin-like growth factor-1 levels also are lower in newborn monkeys that are exposed to tenofovir in utero.<sup>8</sup>

In healthy, normally growing children, bone formation exceeds bone resorption, resulting in increased bone size as well as increased bone mass. Our preliminary findings suggest that the decreased BMD that was seen in our patients was caused by increased bone turnover with bone resorption exceeding bone formation. This is consistent with other studies that have suggested increased bone turnover in HAART-treated children.<sup>4,5,23</sup> Although some patients did seem to have decreased bone in the primary spongiosa, we did not observe rachitic changes as seen in the high-dose animal studies; this may be attributable to the lower dose used in our patients. The reasons for an association of decreased BMD and decreased viral load are not known; however, these findings conceivably could result from higher effective concentrations of TDF, as a result of either higher levels of adherence in the viral load responders or higher intracellular drug concentrations from genetic or other factors. Additional study is necessary to determine whether TDF is acting directly on bone cells or through humoral factors, such as insulin-like growth factors and cytokines, which also regulate bone balance.

This study is limited by its small number of patients and lack of a placebo-controlled study design. Because all of the patients enrolled had severe, multidrug-resistant HIV disease that required a change in therapy and there were no good treatment options that did not involve TDF-containing combination therapy,<sup>11</sup> an untreated control group was not believed to be feasible. Furthermore, antecedent measures of change in BMD could not be obtained because this would delay critical salvage therapy. Given the high dropout rate, it also is uncertain whether BMD loss, in fact, would attenuate in most children over time. Although the lack of a control group or previous BMD measurements limits the validity of this study, the observation that 6 of the patients experienced an absolute decrease in BMD despite adequate linear growth with stabilization over time or improvement after drug discontinuation suggests that these changes were related directly to TDF administration, rather than the underlying disease.

Because the study was designed to obtain needed

pharmacokinetic and safety data for TDF and, at the same time, provide better antiretroviral options for patients whose disease was not responding to antiretroviral therapy, the patients began an optimized background regimen of antiretrovirals 6 days after they began treatment with tenofovir. It therefore is difficult to assign responsibility unequivocally for the changes in BMD to tenofovir. Nevertheless, because patients who discontinued tenofovir while continuing to receive their other drugs showed improvements in BMD, it is likely that tenofovir was responsible for at least some of the observed decreases in BMD. Several patients also were using concurrent inhaled or intranasal steroids during the study. Although even low doses of nonsystemic steroids have been shown to cause bone loss in children,<sup>24</sup> these are not likely to be the primary cause of bone loss in our patients given the recovery that was seen in patients 8 and 17 after discontinuation of TDF alone. Furthermore, 2 of the patients with decreases in BMD were not taking any steroid preparations.

The bone density *z* scores for our patients were calculated using gender- and ethnicity-specific pediatric databases that were generated on different models of Hologic densitometers, using different software than those used in the present study. At the time of this study, no published pediatric databases were derived from the Hologic QDR 4500. Although some studies have attempted to cross-calibrate data from different densitometers,<sup>25</sup> these methods have not been validated in children. Therefore, the *z* scores reported for our patients may not be completely accurate. Nonetheless, the absolute change in BMD compared with baseline indicates that the pattern of decline in *z* score is unlikely to be caused by use of an inadequate reference database.

Several of the patients had short stature or delayed puberty, conditions that have been associated with misinterpretation of pediatric DXA.<sup>26</sup> DXA also has been shown to be less accurate for assessing BMD in HIV-infected children compared with quantitative computed tomography.<sup>27</sup> Furthermore, pubertal staging was assessed by visual inspection of breasts and pubic hair, rather than by palpation of breast tissue and testes, which is more sensitive for assessing gonadarche. Again, the absolute decline in measured BMD compared with baseline despite increases in stature suggests that our findings are not the result of DXA misinterpretation. Interpretation bias is possible because the investigators who interpreted the scans were not blinded in this uncontrolled trial; however, every effort was made to assess scan quality without excessive comparison of previous scans.

## CONCLUSIONS

TDF is a promising antiretroviral agent that may represent an important component of salvage regimens for treatment-experienced children who are infected with

highly resistant HIV. However, the drug should be used with caution in growing children. Although the long-term effects on bone are unknown, careful monitoring (eg, bone densitometry at baseline and every 6–12 months) of HIV-infected children who require treatment with TDF is indicated.

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## Tenofovir Disoproxil Fumarate and an Optimized Background Regimen of Antiretroviral Agents as Salvage Therapy: Impact on Bone Mineral Density in HIV-Infected Children

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