Original article

Effects on vitamin D, bone and the kidney of switching from fixed-dose tenofovir disoproxil fumarate/emtricitabine/efavirenz to darunavir/ritonavir monotherapy: a randomized, controlled trial (MIDAS)

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Background: Efavirenz (EFV) has been associated with reductions in vitamin D (25[OH]D) and tenofovir (TDF) with increased bone turnover, reductions in bone mineral density (BMD) and renal tubular dysfunction. We hypothesized that switching from fixed-dose TDF/emtricitabine (FTC)/EFV to darunavir/ritonavir monotherapy (DRV/r) might increase 25(OH)D and BMD, and improve renal tubular function.

Methods: Subjects with HIV RNA <50 copies/ml on TDF/FTC/EFV for ≥6 months were randomized 1:1 to ongoing TDF/FTC/EFV or DRV/r (800/100 mg once daily) for 48 weeks. The primary end point was change from baseline in 25(OH)D at week 48. Secondary end points included changes in BMD, bone turnover markers and renal tubular function.

Results: A total of 64 subjects (86% male, 66% white, mean [SD] CD4+ T-cell count 537.3 [191.5]/mm^3) were analysed. After adjustment for baseline 25(OH)D and demographics, at week 48 DRV/r monotherapy was associated with a +3.6 (95% CI 0.6, 6.6) ng/ml increase in 25(OH)D compared to TDF/FTC/EFV (P=0.02). DRV/r monotherapy was associated with an increase in BMD (+2.9% versus -0.003% at the neck of femur and +2.6% versus +0.008% at the lumbar spine for DRV/r versus TDF/FTC/EFV; P<0.05 for all) and reductions in bone biomarkers compared with those remaining on TDF/FTC/EFV. No significant difference in renal tubular function was observed. Reasons for discontinuation in the DRV/r arm included side effects (n=4) and viral load rebound (n=3), all of which resolved with DRV/r discontinuation or regimen intensification.

Conclusions: Switching from TDF/FTC/EFV to DRV/r in patients with suppressed HIV RNA resulted in significant improvements in 25(OH)D and bone biomarkers, and a 2–3% increase in BMD.

Introduction

With successful treatment and near-normal life expectancy of people living with HIV [1], the focus of care has shifted towards minimizing toxicity from long-term exposure to antiretrovirals (ARVs) and preventing non-infectious comorbidities such as cardiovascular, bone and renal disease in this ageing population [2]. Exposing patients to fewer classes of ARVs in an attempt to reduce toxicity and treatment costs is appealing. Although not labelled for this use, simplified maintenance therapy with ritonavir-boosted darunavir monotherapy (DRV/r) proved a safe alternative in clinical trials [3,4]. Although low-level viraemia occurred, the high genetic barrier of DRV ensured low levels of resistance-conferring mutations in virological failure [5].

Vitamin D (25[OH]D) plays a major role in bone mineral density (BMD) by maintaining bone health,

calcium and phosphate homeostasis and promoting bone mineralization [6]. An inverse relationship between 25(OH)D and inflammatory and haemostatic markers such as C-reactive protein (CRP), fibrinogen, D-dimer and tissue plasminogen activator [7–10] have been demonstrated and may partly explain the association between 25(OH)D deficiency and an elevated risk of cardiovascular disease, cancer and reduced survival in the general population [11,12]. Traditional risk factors for vitamin D deficiency include reduced exposure to sunlight, winter months, non-Caucasian ethnicity and low dietary intake. In the HIV population, reduced levels of both 25(OH)D and the active form of vitamin D, 1-25-dihydroxy vitamin D ($1,25[OH]_2D$) are reported with efavirenz (EFV) use [13–15]. The clinical relevance of vitamin D deficiency in HIV is yet to be fully defined. 25(OH)D deficiency has been linked to short-term mortality in the EuroSIDA cohort [16] and severe deficiency (25[OH]D <10 ng/l) with low $CD4^+$ T-cell counts <100 cells/μl [17]. Some, but not all, studies describe a similar association between 25(OH)D and inflammatory markers in HIV [17–19]. Increasing 25(OH)D levels through supplementation has a favourable effect on BMD [20] and cellular immune activation levels [21], though less evidence exists for other inflammatory markers associated with cardiovascular risk [22,23].

Tenofovir disoproxil fumarate (TDF)-containing regimes have been associated with greater reductions in BMD in patients initiating TDF, and with BMD reductions in patients who switch ARVs to a TDF-containing regimen [24–26]. Exposure to TDF has also been associated with raised parathyroid hormone (PTH) concentrations [27]. Additional concerns with TDF use include adverse renal outcomes, including acute tubular injury [28], chronic kidney disease [29] and proximal renal tubular dysfunction [30,31].

Studies have demonstrated improved vitamin D levels after switching away from EFV [32] and improvements in BMD and renal function when switching away from TDF [33–35]. We conducted a randomized, controlled clinical trial of a switch from fixed-dose TDF/emtricitabine (FTC)/EFV to DRV/r. We hypothesized that TDF and EFV discontinuation would have favourable effects on vitamin D, PTH, phosphate homeostasis, renal function, bone turnover and BMD.

Methods

Study design

The metabolic impact of DRV/r maintenance monotherapy after successful viral suppression with TDF/FTC/EFV in HIV-positive patients (MIDAS) is a prospective open-label Phase IV randomized controlled trial conducted at two HIV centres in the UK (Guy's and St Thomas' and King's College London NHS Foundation Trusts).

Study population

HIV-1-positive patients aged 18–65 years who were asymptomatic with plasma HIV RNA <100 copies/ml for at least 6 months and agreeable not to take vitamin D supplements during the trial. Exclusion criteria included use of vitamin D supplements in the past 3 months, detectable hepatitis B surface antigen or hepatitis C RNA, hepatic impairment and osteoporosis requiring treatment.

Study intervention

Study participants on TDF/FTC/EFV (300/200/600 mg once daily) were randomized 1:1 to either continue their current regime or to switch to open label DRV/r (800/100 mg once daily) for 48 weeks.

Primary outcome

Change in 25(OH)D from baseline to week 48.

Secondary outcomes

Changes in BMD, $1,25(OH)_2D$, PTH, markers of bone turnover, kidney function and immune activation, and the proportion of participants with two consecutive HIV RNA measurements >50 copies/ml at 48 weeks.

Study procedures

Visits occurred at baseline and weeks 4, 12, 24, 36 and 48. Safety was assessed by physical examination, recording of adverse events, renal, liver, bone and lipid profiles, full blood and $CD4^+$ T-cell count and plasma HIV RNA. Fasted blood and urine samples were collected and frozen at -80° and subsequently analysed for plasma 25(OH)D (Siemens Healthcare Diagnostics Ltd, Erlangen, Germany), $1,25(OH)_2D$, (Immunodiagnostic Systems Ltd [IDS Ltd], Boldon, UK), bone-specific alkaline phosphatase (BAP), PTH, serum cystatin C, type 1 collagen cross-linked C-telopeptide (CTX), procollagen type 1 N-terminal propeptide (P1NP) and urinary phosphate, albumin, protein and retinol binding protein (RBP) concentrations (DELFIA® monoclonal antibody assay, Cambridge, UK [36]). Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration formula (CKD-Epi) for creatinine [37] and for cystatin C [38]. Urinary proteins were expressed as their ratio with urinary creatinine:albumin-creatinine ratio (ACR), protein-creatinine ratio (PCR) and RBP-creatinine ratio (RBPCR). Tubular phosphate handling was described using the ratio of the maximum rate of tubular phosphate reabsorption to the glomerular filtration ($TmPO_4$/GFR) [39].

Dual energy X-ray absorptiometry (DXA) measurements were obtained using the Hologic (Marlborough,

MA, USA) and GE Healthcare (Waukesha, WI, USA) Lunar iDXA bone densitometers at baseline and week 48. Measurements were converted for direct comparison using data from a Bonafide spine phantom. BMD (expressed in g/cm^2) of the lumbar spine (L1–L4 composite), the non-dominant total hip and the non-dominant neck of femur (NOF) were used for analysis.

Sample size calculation

A sample size of 70 patients (35 in each arm) was required to achieve 90% power to detect an increase of 15 nmol/l in 25(OH)D at 48 weeks in the DRV/r arm compared to the TDF/FTC/EFV arm.

Randomization

Potentially eligible patients were screened and reasons for non-entry recorded. Consenting patients were randomised 1:1 using a generated allocation sequence.

Statistical methods

Baseline characteristics were summarized by randomization group as means and standard deviations (continuous normally distributed variables), medians and IQRs (non-normally distributed variables), and frequencies and percentages (categorical variables).

For the primary outcome, the mean difference in 25(OH)D between the two study arms was analysed using linear regression adjusted for baseline 25(OH)D measurements, age, ethnicity, season of recruitment and gender, with robust standard errors. Assumptions were checked graphically. All participants had complete observations at baseline. Missing data at follow-up for the primary outcome were imputed regardless of the reason(s) it was missing with baseline outcomes and explanatory variables used to impute missing data, assuming unobserved measurements were missing at random. Only one subject had missing data at week 48.

Secondary longitudinal outcomes, measured at baseline and weeks 12, 24, 36 and 48 were analysed for all subjects available for analysis using repeated measures mixed effects models with an unstructured variance–covariance matrix to assess mean difference between arms. This method was chosen to allow for correlation between repeated measures, deal with missing data for the secondary outcomes and allow an intention-to-treat analysis. Non-normally distributed variables were transformed to approximate normality. Covariates such as age, ethnicity, and body mass index (BMI) were considered for inclusion in the model. Viral efficacy at week 48 was calculated using an FDA snapshot analysis. All analyses were performed on an intention to treat basis and conducted using STATA (version 12; StataCorp LP, College Station, TX, USA).

Ethical review

The study was approved by the National Research Ethics Service. All subjects provided written informed consent.

Results

Study population

Between October 2011 and October 2012, 70 subjects were randomized to either DRV/r (*n*=36) or TDF/FTC/EFV (*n*=34) and all subjects received the allocated intervention. Prior to week 12, six subjects withdrew from the study, four in the DRV/r arm (due to intolerance to DRV/r [*n*=2], acute HCV coinfection [*n*=1] and patient choice [*n*=1]) and two in the TDF/FTC/EFV arm (due to concomitant vitamin D supplementation; Figure 1). Per protocol, subjects withdrawing prior to week 12 were replaced and not included in the analyses. A total of 64 subjects were included in the primary and secondary analyses; 56 subjects reached week 48, 25 in the DRV/r arm and 31 in the TDF/FTC/EFV arm (Figure 1). Baseline demographics and clinical characteristics were similar between treatment arms (Table 1).

Primary outcome

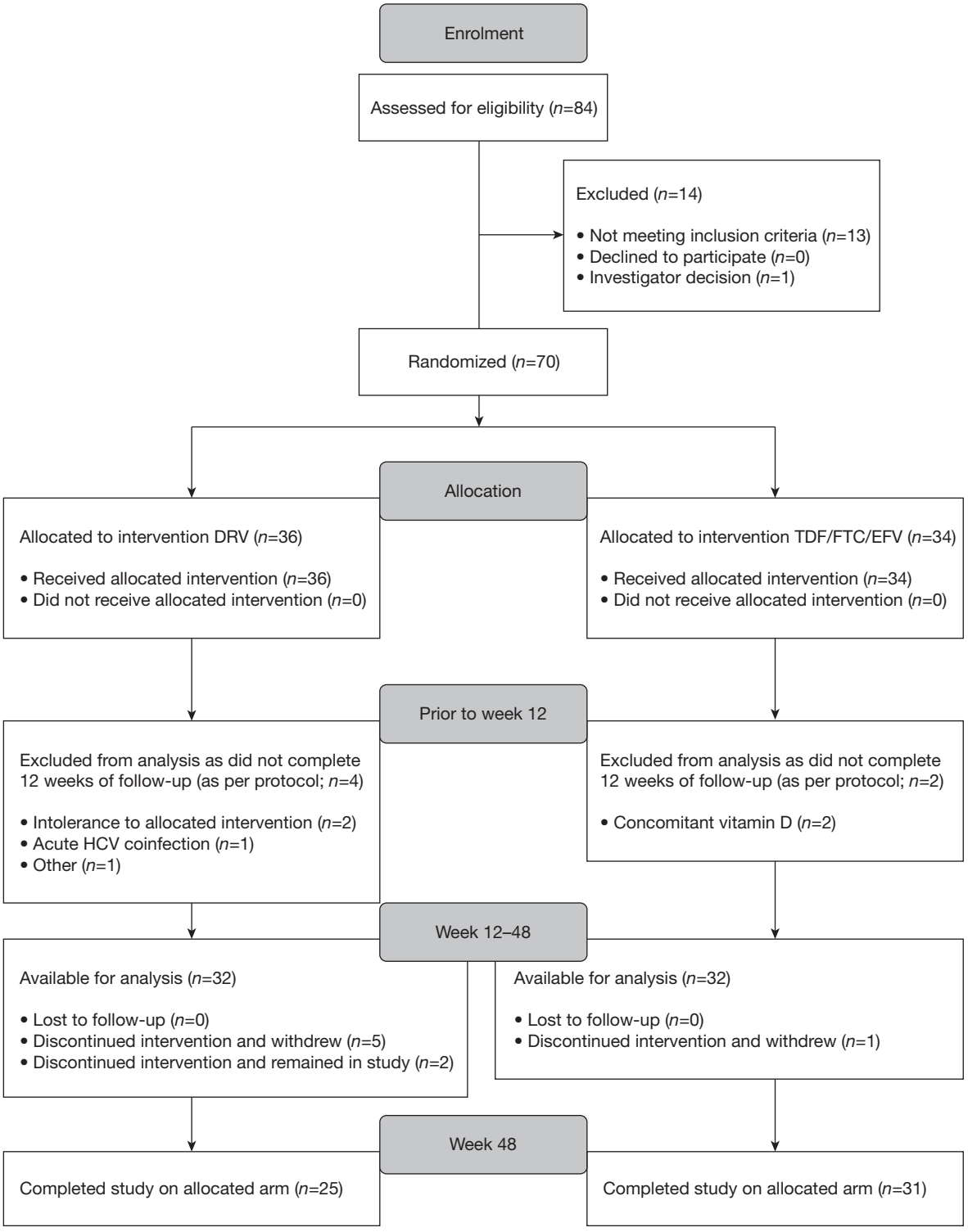
At week 48, 25(OH)D levels were significantly higher in the DRV/r arm compared with the TDF/FTC/EFV arm with a mean adjusted change (95% CI) in 25(OH)D from baseline to week 48 of +3.5 (0.5, 6.4) ng/ml (*P*=0.02) for DRV/r compared with TDF/FTC/EFV. The sensitivity analyses supported the missing at random assumption (data not shown). Using multiple imputation, the effect size did not differ from complete case analysis (Additional file 1), thus complete case analysis is presented here.

Secondary outcomes

1,25(OH)$_2$D concentrations decreased in both arms at week 48, but this was significantly less in the DRV/r arm (mean difference [95% CI] at 48 weeks +12.1 [5.9, 18.3] pmol/l for DRV/r versus TDF/FTC/EFV; *P*<0.001). Change in vitamin D binding protein and PTH concentration did not differ significantly between arms (Table 2).

There was a significant difference in BMD change from baseline to week 48, with BMD improving in the DRV/r arm at all three sites. At week 48, the observed mean percentage BMD change in the DRV/r versus TDF/FTC/EFV arms was +1.8% versus -0.002% at the hip, +2.9% versus -0.003% at the neck of femur and +2.6% versus +0.008% at the lumbar spine (*P*<0.05 for all; Figure 2). These differences remained significant after adjustment for baseline BMD, BMI, age and ethnicity (Table 2). Significant reductions in bone turnover markers (BTMs), including CTX, P1NP and BAP were

Figure 1. Consort diagram



DRV, darunavir; TDF/FTC/EFV, tenofovir disoproxil fumarate/emtricitabine/efavirenz.

Table 1. Demographic and baseline characteristics

	Total (*n*=64)	TDF/FTC/EFV (*n*=32)	DRV/r (*n*=32)
Demographics			
Mean age, years (SD)	42.7 (9.0)	43.8 (9.2)	41.6 (8.8)
Male sex, *n* (%)	54 (84.4)	27 (84.4)	27 (84.4)
Ethnicity			
White/other, *n* (%)	41 (64.1)	22 (68.8)	19 (59.4)
Black, *n* (%)	23 (35.9)	10 (31.3)	13 (40.6)
Current smoker, *n* (%)	15 (23.8)	9 (28.1)	6 (19.4)
Mean weight, kg (SD)	77.2 (13.0)	77.7 (13.8)	76.6 (12.3)
Median BMI, kg/m^2 (IQR)	24.8 (22.2–27.8)	25.3 (22.1–29.3)	24.7 (23.1–27.1)
Mean BMD Z-score hip (SD)	-0.05 (0.9)	-0.04 (0.9)	-0.05 (0.9)
HIV parameters			
HIV transmission risk			
MSM, *n* (%)	43 (67.2)	23 (71.9)	20 (62.5)
Heterosexual, *n* (%)	21 (32.8)	9 (28.1)	12 (37.5)
Median years since HIV diagnosis (IQR)	6.4 (3.9–10.1)	7.1 (4.0–9.5)	6.2 (3.9–10.4)
Prior AIDS diagnosis, *n* (%)	11 (18.0)	5 (17.2)	6 (18.8)
CD4$^+$ T-cell count			
At recruitment, mean (SD)	535.1 (194.2)	544.7 (175.0)	525.6 (214.1)
Nadir, median (IQR)	178.5 (73.5–275.5)	178.5 (50.5–252)	189.5 (125–319)
Median years on TDF/FTC/EFV (IQR)	3.5 (2.5–3.9)	3.5 (2.4–4.0)	3.3 (2.5–3.8)

BMD, bone mineral density; BMI, body mass index; DRV/r, ritonavir-boosted darunavir; EFV, efavirenz; FTC, emtricitabine; MSM, men who have sex with men; TDF, tenofovir disoproxil fumarate.

observed following a switch to DRV/r; these differences remained significant after adjustment for age, ethnicity, years on TDF/FTC/EFV and baseline 25(OH)D concentration ($P<0.05$ for all; Table 2). No correlation between baseline 25(OH)D and BTMs or BMD ($P>0.1$ for all) was observed. Early changes in 25(OH)D and PTH (in the first 4 weeks) were weakly correlated with change in BMD at the spine (r^2 0.3; $P=0.05$ and r^2 -0.3; $P=0.05$) but these failed to reach statistical significance. No other early changes in bone or vitamin D parameters were observed.

There were no significant differences between arms for any renal outcomes including eGFR calculated with either CKD-Epi-creatinine or CKD-EPI-cystatin C, ACR, PCR, log RBPCR and $TmPO_4$/GFR (Table 2).

Adverse events

During the study period, 258 AEs were reported in 61/64 (95.3%) subjects with no difference between arms (50.8% DRV/r versus 49.2% TDF/FTC/EFV; $P=0.55$). In the DRV/r arm diarrhoea, coryzal symptoms and cutaneous rash were the most commonly reported AE (8.4, 5.8 and 4.5%, respectively) and in the TDF/FTC/EFV arm, infection (upper respiratory tract infection), headache and cutaneous rash were the most common (11.4, 7.0 and 7.0%, respectively). All non-serious (grade 2) AEs are described in Additional file 2.

There were six reported serious adverse events (SAEs), of which four were severe and two were moderate in intensity (Additional file 3). In the TDF/FTC/EFV arm, one subject experienced concussion and one subject presented with left anterior uveitis. Neither was felt to be related to TDF/FTC/EFV and no action with the study drug was taken. One subject on TDF/FTC/EFV experienced secondary hyperparathyroidism which was felt to be likely to be related to TDF/FTC/EFV, which was subsequently permanently withdrawn.

In the DRV/r arm, none of the three SAEs which included meningoencephalitis, *Mycobacterium kansasii* infection of the hip or seizure with a pre-existing seizure disorder were felt to be related to the study drug. The subject with meningoencephalitis presented with severe headache and dizziness and was determined to have a cerebrospinal fluid (CSF) HIV RNA viral load of 9,450 copies/ml with a plasma HIV viral load of 217 copies/ml. The study drug was permanently withdrawn and the subject switched back to TDF/FTC/EFV and symptoms completely settled. There were six further discontinuations from the allocated intervention in the DRV/r arm: three due to HIV viral load rebound (Additional file 4) all had their ART regime intensified (*n*=2) or switched back to TDF/FTC/EFV (*n*=1) and HIV RNA became fully supressed. No virological resistance was documented. The full FDA snapshot algorithm is presented in Table 3. Two subjects experienced side effects including gastrointestinal symptoms and darkening of skin and one complained of the increased pill burden; all switched back to TDF/FTC/EFV.

Table 2. Study outcomes (ITT analysis)

Secondary outcomes	Baseline (mean [SD] or median [IQR])[a] TDF/FTC/EFV	Baseline (mean [SD] or median [IQR])[a] DRV/r	Week 48 (mean [SD] or median [IQR]) TDF/FTC/EFV	Week 48 (mean [SD] or median [IQR]) DRV/r	Adjusted mean difference (95% CI) between arms from baseline to week 48[b]	*P*-value
Bone outcomes						
25(OH)D, ng/ml	12.3 (6.3–19.1)	11.3 (6.2–17.7)	12.3 (8.3–21)	16.2 (11.8–23.3)	**3.6 (0.6, 6.6)**	**0.02**
1,25(OH)D, pmol/l	88.2 (58.8–108.2)	88.7 (62.3–115.5)	63.9 (57.3–86.2)	76.4 (52.8–99.1)	**12.1 (5.9, 18.3)**	**<0.001**
Vitamin D binding protein, mg/l	208.0 (107.6–325.9)	189.3 (115.9–265.0)	203.3 (98.6–329.5)	203.7 (90.2–282.7)	4 (-5.2, 13.2)	0.4
PTH, ng/l	47.4 (34.3–71.4)	47.0 (35.5–74.5)	51.0 (36.0–68.0)	50.0 (28.9–64.0)	-7.7 (-19.1, 3.8)	0.2
Serum calcium, mmol/l	2.2 (0.1)	2.2 (0.1)	2.2 (0.1)	2.2 (0.1)	0.02 (-0.01, 0.05)	0.2
Serum phosphate, mmol/l	1.0 (0.2)	1.0 (0.2)	1.0 (0.1)	0.9 (0.1)	-0.05 (-0.1, 0.002)	0.06
Serum ALP, IU/l	92 (79–112)	78 (63–104)	95 (76–106)	60 (48–67)	**-34.3 (-44.9, -23.6)**	**<0.001**
Bone-specific ALP, IU/l	26.1 (7.0)	23.9 (6.0)	26.4 (6.4)	18.2 (5.0)	**-5.5 (-8.4, -2.6)**	**<0.001**
CTX, µg/l	0.4 (0.2)	0.3 (0.1)	0.37 (0.18)	0.25 (0.13)	**-0.1 (-0.2, -0.06)**	**<0.001**
P1NP, µg/l	53.2 (20.5)	52.0 (16.5)	55.9 (17.6)	36.0 (10.6)	**-9.5 (-18.5, -0.5)**	**0.04**
BMD						
Total hip, g/cm^2	1.01 (0.14)	1.02 (0.15)	1.01 (0.14)	1.06 (0.17)	**0.02 (0.0005, 0.03)**	**0.04**
Neck of femur, g/cm^2	0.92 (0.17)	0.90 (0.16)	0.91 (0.15)	0.94 (0.19)	**0.03 (0.005, 0.05)**	**0.02**
Spine, g/cm^2	1.09 (0.17)	1.11 (0.18)	1.10 (0.17)	1.14 (0.21)	**0.03 (0.003, 0.05)**	**0.02**
Renal outcomes						
eGFR						
CKD-Epi, ml/min/1.73 m^2	105.4 (14.6)	109.5 (15.6)	104.4 (14.8)	99.6 (14.7)	-1.0 (-6.6, 4.6)	0.7
Cystatin C, ml/min/1.73 m^2	114.0 (20.2)	115.6 (22.3)	110.7 (22.1)	111.3 (21.6)	-3.2 (-12.1, 5.7)	0.5
ACR, mg/mmol	0.4 (0.3–0.8)	0.5 (0.3–0.6)	0.4 (0.3–0.7)	0.4 (0.3–0.8)	-0.8 (-2.3, 0.6)	0.2
PCR, mg/mmol	7.3 (5.3–11.5)	6.2 (0.9–8.7)	8.6 (6.8–11.3)	7.0 (5.7–10.8)	-2.4 (-5.7, 0.9)	0.2
$TmPO_4$/GFR, mmol/l	1.0 (0.2)	1.0 (0.2)	1.0 (0.2)	0.9 (0.2)	-0.02 (-0.4, 0.4)	0.9
RBPCR, µg/mmol	2.1 (1.8–4.5)	2.2 (1.5–4.4)	1.9 (1.2–3.1)	2.0 (1.3–2.8)	-0.04 (-0.1, 0.04)	0.3

Bold represents statistical significance. [a]There were no statistically significant differences between arms for all baseline parameters. [b]Adjusted for age, ethnicity, HIV transmission risk, viral load, CD4$^+$ T-cell count, years diagnosed with HIV, years on tenofovir disoproxil fumarate/emtricitabine/efavirenz (TDF/FTC/EFV) and body mass index. ACR, urinary albumin/creatinine ratio; ALP, alkaline phosphatase; BMD, bone mineral density; CKD-Epi, Chronic Kidney Disease Epidemiology Collaboration; CTX, type 1 collagen cross-linked C-telopeptide; DRV/r, ritonavir-boosted darunavir monotherapy; eGFR, estimated glomerular filtration rate; PCR, urinary protein/creatinine ratio; PTH, parathyroid hormone; P1NP, procollagen type 1 N-terminal propeptide; RBPCR, urinary retinol binding protein-4/urinary creatinine ratio; $TmPO_4$/GFR, the ratio of the maximum rate of tubular phosphate reabsorption to the glomerular filtration rate; 25(OH)D, 25-hydroxy vitamin D; 1,25(OH)D, 1,25-hydroxy vitamin D.

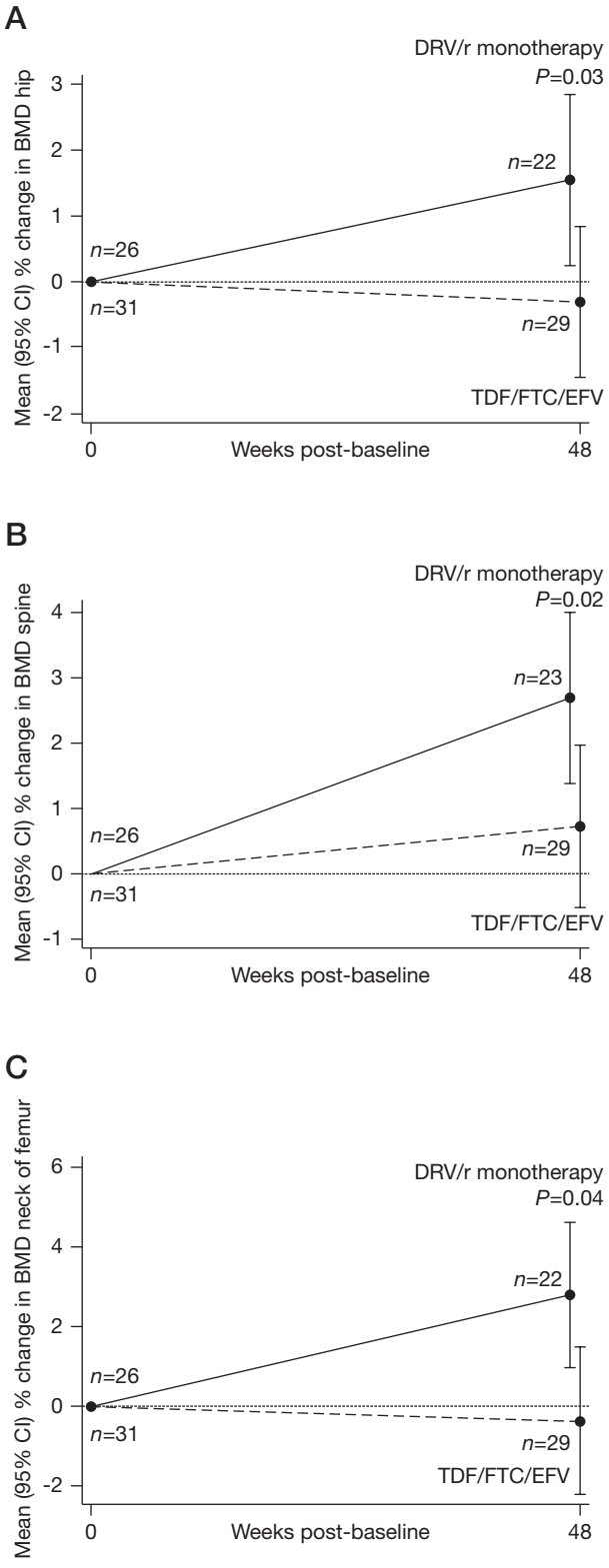
Discussion

This study demonstrates that patients on TDF/FTC/EFV with well-controlled HIV who switched to DRV/r experienced significant improvements in 25(OH)D, $1,25(OH)_2D$ and BMD at the hip, spine and neck of femur. By contrast, no change was observed in PTH, eGFR or renal tubular biomarkers, and one-third of patients who switched to DRV/r required treatment modification due to intolerance or viral rebound.

An increase in plasma vitamin D concentration after discontinuation of EFV and a switch to a protease inhibitor (PI)-based regimen is consistent with data from observational studies that link vitamin D deficiency to EFV exposure [13] and data from the MONET trial in which patients switched from non-nucleoside reverse transcriptase inhibitor (NNRTI)- or PI-based combination antiretroviral therapy (cART) to either DRV/r or DRV/r plus two NRTIs [32]. *In vitro* studies demonstrate EFV reduces expression of CYP2R1, which is involved in 25(OH)D production [40] and induces CYP24, which catabolizes 25(OH)D and $1,25(OH)_2D$ into inactive metabolites [41]; subsequent removal of EFV could be postulated to reverse these effects. We failed to observe an increase in $1,25(OH)_2D$ in the DRV/r arm, which may, in part, be attributed to *in vitro* evidence which suggests that PIs inhibit 1α-hydroxylase, the renal enzyme which converts 25(OH)D into the active $1,25(OH)_2D$ [42].

Initiation of ARVs has been associated with BMD reductions of up to 6% in the first year of treatment [24,43,44]. However, in our study, a switch from TDF/FTC/EFV to DRV/r was associated with a 2–3% increase in BMD across all sites and a reduction in bone turnover. This is consistent with data from the TROP study in which 37 patients substituted TDF with raltegravir (RAL) [33], the Monarch study in which 15 patients on cART (93% on TDF, 20% already on a PI) switched to DRV/r [45] and a Spanish study in which 26 patients changed from TDF to abacavir [35]. Bone protective antiretroviral strategies in which TDF is replaced by RAL or the entire regimen is replaced by DRV/r may

Figure 2. Percentage change in BMD at 48 weeks



(A) Hip. **(B)** Spine. **(C)** Neck of femur. BMD, bone mineral density; DRV/r, ritonavir-boosted darunavir; TDF/FTC/EFV, tenofovir disoproxil fumarate/emtricitabine/efavirenz.

be an attractive alternative to vitamin D plus calcium supplementation (associated with approximately 1% BMD increase) or bisphosphonate therapy (2–4%, and in some studies up to 8% BMD increase) [46–49].

We observed a 24% and 32% reduction in CTX and P1NP, respectively, in the DRV/r arm. Response to therapy with bisphosphonates is considered significant if associated with a decrease in BTMs of 30% or more from baseline and one large study in postmenopausal women with newly diagnosed osteoporosis suggested reductions of BTMs of this magnitude are associated with decreased fracture risk [50].

The relative contribution of increased 25(OH)D to the observed improvement in BMD is hard to quantify. We demonstrate a weak correlation between an early increase in 25(OH)D and decrease in PTH with improved BMD at week 48. A placebo controlled trial of vitamin D3 and calcium supplementation in treatment-naive patients starting TDF/FTC/EFV demonstrated attenuation of bone loss at the hip for vitamin D versus placebo (-1.36 versus -3.19%; *P*=0.001), although the increase in 25(OH)D was greater in magnitude than our study (median [IQR] change 24.5 [14.6–37.7] ng/ml) [20].

We allowed for the effect of season on vitamin D levels by using the difference between baseline and week 48. Adjusting for season in the analysis did not change the effect size, nor was season found to be an effect modifier (data not shown). In contrast to Fabre-Mersseman *et al.* [21], we observed no improvement in inflammatory markers. This may be in part due to the relatively small increase in 25(OH)D in this study or our small sample size of well patients on stable ART.

The mechanism of TDF-associated bone loss is not fully elucidated. Uptake of TDF into osteoclasts or osteoblasts, causing cellular stress and perturbed DNA synthesis [51] or altered gene expression [52,53] could result in reduced BMD. TDF has also been associated with proximal renal tubular dysfunction and the accompanying renal phosphate wasting and osteomalacia may be a proposed mechanism [51]. The BMD increase in the DRV/r arm of our study was not explained by changes in 25(OH)D, PTH or renal tubular function, suggesting that direct effects of TDF on bone may have affected baseline BMD in our patients.

We observed no change in any of the renal biomarkers. The lack of change in cystatin C based eGFR suggests that glomerular function was unaffected by the switch from TDF/FTC/EFV to DRV/r. This may be explained by the patient population in that only those with preserved and stable renal function on TDF/FTC/EFV were enrolled. Furthermore, some improvement in eGFR in patients who switched to DRV/r may have been countered by ritonavir-induced MATE-1 inhibition of tubular creatinine secretion [54]. The lack of change in tubular function is

Table 3. FDA snapshot analysis of virological outcomes at week 48

	DRV/r	TDF/FTC/EFV
HIV RNA<50 copies/ml	21 (65.6)	30 (93.8)
HIV RNA≥50 copies/ml[a]	7 (21.9)	1 (3.1)
No virological data at week 48 window	4 (12.5)	1 (3.1)
Discontinued study/study drug due to AE[b]	2 (6.3)	1 (3.1)
Discontinued study/study drug due to other reasons[c]	2 (6.3)	0
On study but missing data in window	0	0

Data are *n* (%). [a]Includes patients who changed any component of background therapy to a new drug class or changed background components that were not permitted per protocol or changed any background drug in the regimen because of lack of efficacy (perceived or documented) before week 48, patients who discontinued study drug or study before week 48 for lack or loss of efficacy and patients who are equal to or above 50 copies/ml in the 48 week window. [b]Includes patients who discontinued because of adverse event (AE) or death at any time point from day 1 through the time window if this resulted in no virological data on treatment during the specified window. [c]Other includes: withdrew consent, loss to follow-up, moved, among others. DRV/r, ritonavir-boosted darunavir; TDF/FTC/EFV, tenofovir disoproxil fumarate/emtricitabine/efavirenz.

somewhat unexpected but may be explained by the lesser perturbation of renal tubular function in patients who receive TDF with an NNRTI as compared with a boosted PI [31]. Moreover, while crystalluria has been reported in 7.8% of DRV/r recipients [55], the effect of DRV/r on renal tubular function has not been described.

Self-reported adherence to the study drugs was excellent; however, there was an excess of viral load rebound and one case of CSF viral escape in the DRV/r arm. Similar to other PI monotherapy studies, we demonstrate rapid return to virological control, low risk of emerging PI resistance and no loss of future treatment options [3–5].

Limitations of this study include the relatively short follow-up, the small sample size, the high rate of DRV/r discontinuation and the lack of recruitment of the required 70 subjects, limiting the power of the study to detect a significant difference. We originally based our power calculation on a difference of 6 ng/ml between arms; to achieve 90% power we required 70 patients in total. Although we recruited 64 patients and observed an absolute difference of 3 ng/ml between arms, the trial only had 71% power for the primary outcome. Although we demonstrate improvements in vitamin D, bone turnover and BMD at the hip and lumbar spine, we cannot decipher whether changes were due to withdrawal of TDF or EFV and the short follow-up time precludes predictions of long-term BMD change or its clinical significance in terms of reduced fracture risk.

In summary, while not always well-tolerated or successful virologically, PI monotherapy may be an attractive strategy for patients on a TDF- and EFV-containing regimen who have developed osteoporosis or fractures and for those at greatest risk of developing these complications.

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LH, FAP and JF designed the trial. LH, JMT, HI, MT, EW, IJ, CT, RK, AT, JF and FAP conducted the trial visits and were responsible for data acquisition, CM and JC provided biobanking support, and KB conducted the RBP analyses. LH was the trial statistician and wrote the statistical analysis plan and undertook the main data analysis under supervision of FI, the senior trial statistician, LH drafted the paper with input from FAP and JF. All authors provided input and approved the final draft of the manuscript.

This study has been presented in abstract form at the *21st Annual Conference of the British HIV Association*, Brighton, UK, 21–24 April 2015.

The lead author affirms that this manuscript is an honest, accurate and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

Anonymized patient level data and statistical analysis plan will be available from the corresponding author for inclusion in meta-analyses and other academic endeavours.

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Additional files

Additional file 1: A table of primary outcome using multiple imputation can be found at http://www.intmedpress.com/uploads/documents/3622_Hamzah_Addfile1.pdf

Additional file 2: A table of all DAIDS grade 2 adverse events and relationship to study drug can be found at http://www.intmedpress.com/uploads/documents/3622_Hamzah_Addfile2.pdf

Additional file 3: A table of serious adverse events and relationship to study drug can be found at http://www.intmedpress.com/uploads/documents/3622_Hamzah_Addfile3.pdf

Additional file 4: A table of virological rebounds can be found at http://www.intmedpress.com/uploads/documents/3622_Hamzah_Addfile4.pdf

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