FINAL STUDY REPORT

Study Title: Effects of Vitamin D supplementation on Vitamin D levels and immune activation in HIV infected individuals on antiretroviral therapy-A pilot study.

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List of Publications (or plans for publications) including those for patients (if applicable)	1) No Benefit of Standard Vitamin D/calcium Supplementation HIV-infected patients (planned for submission to HIV medicine) 2) Abstract has been submitted to The annual Conference on Retroviruses and Opportunistic Infections (CROI) in September 2015
Study Start and End Dates	Start: 3rd October 2011 End: 10/04/2014
Study Design	Prospective open label phase IV study, in which patients stable on antiretroviral therapy with confirmed vitamin D deficiency (25[OH] D <40 nmol/L or 16nng/ml) were randomised 1:1 to receive vitamin D3/calcium carbonate supplementation (800 IU/3000 mg) or no treatment (control arm) for 48 weeks.
No. of Patients (planned and analysed)	30 subjects were randomised to either supplementation (n=15) or no supplementation (n=15). 1 subject withdrew from the study before week 12. 29 patients were analysed.
Main inclusion/exclusion criteria	*Inclusion criteria* • Male and female • HIV infected patients on antiretroviral therapy • Between 18-65 years of age • CD4 >350

	• Vitamin D deficiency [25(OH) vit D <40 nmol/L] • Females must be willing to use barrier method contraception for the duration of the study *Exclusion criteria* • HIV infected individuals not receiving antiretroviral therapy • Patients with normal Vitamin D levels (> 40 nmol/L) • Patients taking Vitamin D supplements • Documented soya or peanut allergy • Patients unable to provide written consent or comply with protocol • Pregnancy or trying to become pregnant • Confirmed Osteopenia or osteoporosis Participation on any other interventional study
Investigational Medicinal Product(s) (including comparator, if applicable), mode of administration and batch number(s)	Adcal D3® lemon flavoured chewable tablet: 2 tablets daily (either as single or split dose);. Manufacturer: Pro Straken. (tablet contains: Calcium carbonate 1.5g, cholecalciferol 10 micrograms) All drugs were supplied by the local site pharmacy from normal clinic stock in standard packaging. Drug was be re-labelled in the local study site pharmacy with study information and prescribed using a trial specific prescription.
Duration of Treatment	48 weeks follow-up after randomisation
Primary and Secondary Objective(s)	**Primary aim:** To investigate the role vitamin D supplementation on restoring vitamin D levels in HIV infected individuals on antiretroviral therapy. **Secondary Aims:** To investigate the effect of vitamin D on: 1. Immune activation (CD8 CD38) 2. Surrogate markers of clinical outcome (CD4 and HIV RNA) 3. Active Vitamin D (1,25OH) 4. Markers of coagulation and inflammation [CRP] 5. Parathyroid hormone levels
Endpoints/ Outcome Measure(s)	**Primary Outcome** • Change in 25(OH)D at week 48 **Secondary Outcome:** • Improvement in bone mineral density (BMD) • Immune activation: change in immune activation ($CD8^+CD38^+$) • Surrogate markers of clinical outcome (CD4 and HIV RNA) • active Vitamin D (1,25OH) • Markers of coagulation and inflammation [CRP] • Parathyroid hormone levels • T cell immune function

Statistical Methods	The primary outcome is the change in 25 (OH) D level at 48 weeks from baseline. There is currently no established data from which to estimate values from, therefore this study was designed as a pilot in order to determine the natures of the differences and inform further studies. Thus no formal power calculation was undertaken. 30 patients were recruited, with a 1:1 randomisation, 15 patients per arm and followed up for 48 weeks. Due to seasonal variation in vitamin D levels, the difference between baseline and week 48 25(OH) D levels was considered to ensure both measurements were taken during the same season to minimise biasing the results. For this and other single outcome measures, linear regression was used to analyse mean difference between arms at 48 weeks, adjusted for baseline measurements, age, ethnicity and gender. Primary outcome data was available for all patients, only two patients were missing secondary measures and for these data we used last observation carried forward. Since the primary analysis is likelihood based and assumed data was missing at random (MAR). Secondary longitudinal outcomes were analysed using repeated measures mixed effects models with an unstructured variance-covariance matrix to assess mean difference between arms. The impact of age, ethnicity and gender were assessed including in the final model. Interaction between allocated arm and visit were investigated. All analyses were based on an intention-to-treat basis to reflect the randomisation process, except for enrolled patients who were found to be ineligible or who withdraw consent at baseline visit and so never receive any treatment. All statistical tests were two-tailed. P-values and/or confidence intervals were presented as appropriate. All analyses were performed on an intention to treat basis and conducted using STATA statistical software (version 14).
Conclusions	30 subjects, 80% male in the vitamin D arm and 86% in the control arm respectively were randomized. Age (SD) was 43.5 years, 72 % of individuals were white. 13 (45%) of patients were receiving a PI based regimen, 14(48%) were on NNRTIs and 2(7%) received integrase inhibitors. One patient in the control arm was lost of follow up early after recruitment. Overall baseline 25 [OH] D (SD) was 15.89 nmol/L (9.78). **Primary endpoint** There was no difference between baseline 25(OH)D levels (mean [SD] supplementation and control respectively. At week 48, the unadjusted mean [SD] change from baseline in 25(OH)D was -5.4 nmol/Lin the supplementation arm compared to +0.6 nmol/Lin the control arm (p=0.069) for the supplementation arm compared to control. **Secondary endpoints** ***Bone outcomes*** At week 48, the unadjusted mean percentage change in bone density was -.0.01% vs. -0.04% (p=0.920) at the hip, and -0.13% vs. 0.1% (p=0.918) at the spine for supplementation vs. control, p>0.05 for all. There was no difference between arms in parathyroid hormone (-12.3 v -9.6 ng/l p=0.75), serum calcium (+0.1 v +0.1 p=0.365), serum phosphate levels (-0.1 V 0mmol/L p=0.953) or serum ALP (-3.8 v +0.8mmol/L p=0.124), vitamin d binding protein (+13.5 v +13.6mmol/L p=0.998). Multivariate analysis found no association between low vitamin D level

	and efavirenz based regiment (p=0.50) or ethnicity (p=0.20) ***Immunological outcomes*** There was no significant difference between arms in terms of CD4 change or proportion with an undetectable viral load (p=0.222) ***Adverse events*** During the study period, a total of 29 AEs were reported in n=15 subjects on supplementation and 14 control subjects and where not related to intervention group. All the events have resolved. One patient had serious adverse events. There were no difference in terms of adverse event between the intervention group (Fisher's exact test p=0.215). In both arms infection/allergy was the most commonly reported AE (44% v 50%).

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