Vitamin D does not modulate immune-mediated bone loss during ART initiation: A5280

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Background

- Bone loss of 2-4% observed with ART initiation may be due to direct effects of ART on bone cells or to pro-resorptive inflammatory cytokines released with immune reconstitution.

- Activated T cells express TNFα, IL6 and receptor activator of nuclear factor kappa-B ligand (RANKL), which induce osteoclast activation and differentiation, resulting in bone resorption.

- Vitamin D (VitD) downregulates cytokine release in activated T cells and suppresses T cell proliferation. Therefore, VitD may play a role in reducing inflammation and mitigate immune-mediated bone loss during ART initiation.

- A5280 was a randomized, double-blind, placebo-controlled, 48-week study of Vitamin D and calcium supplementation (VitD3 4000 IU & CaCO$_3$ 1000 mg daily) with initiation of efavirenz/emtricitabine/tenofovir DF (EFV/FTC/TDF). VitD/Ca supplementation attenuated decreases in bone mineral density (BMD) at the total hip and lumbar spine by approximately 50% compared to placebo

Objective

In this exploratory analysis, we examined the effect of VitD/Ca supplementation on cellular immune phenotypes and BMD.

We hypothesized that VitD supplementation would result in decreases in cellular immune activation markers which would also be associated with less bone loss during ART initiation.

Methods

Cellular phenotype methods

- Stored peripheral blood mononuclear cells (PBMCs) were thawed and surface stained with fluorochrome-conjugated monoclonal antibodies to CD3, CD4, CD8, CD19, CD28, CD38, CD57, HLA-DR, CD25, and RANKL, stained intracellularly for FoxP3, or OPG Biotin and Streptavidin-BV421, washed and fixed in 1% formaldehyde.

- PBMCs acquired on a LSRFortessa flow cytometer and analyzed using FlowJo software (TreeStar, version 9.9.3).

- Immune activation (CD38+/HLA DR+), senescence (CD28-/CD57+), were characterized on CD4+ and CD8+ T cell subsets. Treg (CD4+/CD25+/FoxP3+) and CD19+ B cells were analyzed. Results presented as % of parent population for immune phenotypes and compared.

- Expression of RANKL and OPG was measured by Mean Fluorescence Intensity (MFI) in CD4+ and CD8+ T cell subsets and CD19+ B cells.

Statistical Methods

- ITT approach limited to participants with available data regardless of treatment change/discontinuation.

- All statistical tests are 2-sided at the 5% nominal level of significance without adjustment for multiple testing.

Table 1. Characteristics at Baseline (N=138)

		Total (N=138)	Calcium/Vit.D (N=64)	Placebo (N=74)
Age (years)	Median (Q1 - Q3)	32 (25 - 45)	33 (25 - 47)	31 (25 - 44)
Race/Ethnicity	White Non-Hispanic	56 (41%)	26 (41%)	30 (41%)
	Black Non-Hispanic	43 (31%)	19 (30%)	24 (32%)
	Hispanic (Regardless of Race)	31 (22%)	16 (25%)	15 (20%)
	Asian, Pacific Islander	6 (4%)	1 (2%)	5 (7%)
	American Indian, Alaskan Native	2 (1%)	2 (3%)	0 (0%)
Sex	M	127 (92%)	59 (92%)	68 (92%)
Entry HIV-1 RNA (log$_{10}$ copies/mL)	Median (Q1 - Q3)	4.5 (4.0 - 5.0)	4.5 (4.1 - 5.0)	4.5 (4.0 - 5.0)
Entry CD4 count (cells/mm³)	Median (Q1 - Q3)	350 (252 - 500)	350 (263 - 514)	343 (245 - 480)
Entry BMI (kg/m²)	Median (Q1 - Q3)	24.3 (21.9 - 28.0)	24.8 (22.4 - 28.4)	23.9 (21.4 - 27.2)
Screening Vitamin D level (ng/mL)	Median (Q1 - Q3)	24 (18 - 34)	26 (20 - 37)	23 (17 - 28)
BMD at hip (g/cm²)	Median (Q1 - Q3)	1.05 (0.96 - 1.19)	1.09 (0.96 - 1.19)	1.03 (0.95 - 1.13)
BMD at spine (g/cm²)	Median (Q1 - Q3)	1.12 (1.02 - 1.23)	1.15 (1.04 - 1.26)	1.09 (0.99 - 1.21)
Z score for BMD at hip	Median (Q1 - Q3)	0.00 (-0.80 - 0.50)	0.00 (-0.80 - 0.50)	-0.15 (-0.80 - 0.40)
Z score for BMD at spine	Median (Q1 - Q3)	-0.30 (-1.20 - 0.50)	0.00 (-0.75 - 0.65)	-0.55 (-1.30 - 0.40)
IL-6 (log$_{10}$ pg/mL)	Median (Q1 - Q3)	0.09 (-0.09, 0.28)	0.07 (-0.09, 0.28)	0.13 (-0.09, 0.28)
sTNFr-1(log$_{10}$ pg/mL)	Median (Q1 - Q3)	3.04 (2.98, 3.11)	3.04 (2.98, 3.12)	3.05 (2.98, 3.10)
sTNFr-2(log$_{10}$ pg/mL)	Median (Q1 - Q3)	3.57 (3.45, 3.67)	3.57 (3.44, 3.66)	3.59 (3.47, 3.67)
sCD14 (log$_{10}$ ng/mL)	Median (Q1 - Q3)	3.16 (3.08, 3.29)	3.15 (3.07, 3.28)	3.19 (3.10, 3.29)

Results

Table 2. Baseline and change in cellular phenotypes and expression of RANKL and OPG on lymphocytes by Median Fluorescence Intensity (MFI) from 0-48 weeks by treatment arm

Cellular Marker	Treatment Arm	N	Baseline Median (Q1, Q3)	Change Median (Q1, Q3)	95% CI for Change Median	p-value (within group)[a]	p-value (between group)[b]
% CD38+/HLA-DR+(CD4+)	Calcium/Vit.D	64	6.8 (3.8, 12.8)	-3.0 (-5.8, -0.9)	(-3.5, -1.6)	<0.001	0.29
	Placebo	74	8.3 (4.6, 11.1)	-3.6 (-7.0, -1.1)	(-4.9, -2.6)	<0.001	
% CD28-/CD57+(CD4+)	Calcium/Vit.D	64	4.0 (1.1, 12.3)	-0.8 (-3.6, -0.0)	(-1.9, -0.3)	<0.001	0.69
	Placebo	74	3.7 (1.7, 13.0)	-1.0 (-5.1, -0.0)	(-2.5, -0.3)	<0.001	
% Treg+(CD4+)	Calcium/Vit.D	64	4.2 (2.9, 5.7)	-0.1 (-1.2, 0.4)	(-0.6, 0.2)	0.083	0.50
	Placebo	74	3.9 (2.7, 5.3)	-0.4 (-1.3, 0.1)	(-0.8, -0.1)	0.002	
% CD38+/HLA-DR+(CD8+)	Calcium/Vit.D	64	18.0 (12.3, 33.0)	-13.9 (-23.2, -9.1)	(-16.0, -11.4)	<0.001	0.77
	Placebo	74	22.8 (12.5, 32.2)	-17.2 (-25.8, -7.7)	(-20.7, -11.4)	<0.001	
% CD28-/CD57+(CD8+)	Calcium/Vit.D	64	30.3 (22.1, 40.2)	14.7 (7.1, 23.1)	(9.4, 18.4)	<0.001	0.56
	Placebo	74	29.2 (18.6, 41.3)	13.0 (6.5, 19.8)	(9.0, 15.1)	<0.001	
% CD38+/HLA-DR+(CD19+)	Calcium/Vit.D	63	94.0 (88.0, 97.4)	-2.0 (-5.1, -0.3)	(-3.1, -1.3)	<0.001	0.27
	Placebo	73	92.5 (86.2, 95.9)	-3.1 (-6.7, -0.1)	(-4.7, -1.9)	<0.001	

MFI	Treatment Arm	N	Baseline Median (Q1, Q3)	Change Median (Q1, Q3)	95% CI for Change Median	p-value (within group)[a]	p-value (between group)[b]
RANKL+(CD4+) MFI	Calcium/Vit.D	63	1223 (1126, 1415)	3 (-81, 119)	(-35, 38)	0.70	0.76
	Placebo	73	1215 (1081, 1485)	10 (-86, 101)	(-58, 46)	0.83	
OPG(CD4+) MFI	Calcium/Vit.D	63	11255 (5876, 20677)	-830 (-7836, 483)	(-3454, -145)	0.003	0.53
	Placebo	73	11312 (5803, 22868)	-2391 (-6844, 103)	(-5116, -977)	0.002	
RANKL+(CD8+) MFI	Calcium/Vit.D	63	1226 (1112, 1453)	-18 (-108, 135)	(-47, 30)	0.90	0.56
	Placebo	73	1268 (1118, 1477)	-15 (-113, 91)	(-46, 22)	0.32	
OPG(CD8+) MFI	Calcium/Vit.D	63	16612 (6689, 28473)	-1451 (-10341, 765)	(-5638, -181)	0.004	0.65
	Placebo	73	18510 (8396, 32957)	-3067 (-10567, 327)	(-7147, -708)	0.003	
RANKL+(CD19+) MFI	Calcium/Vit.D	63	8230 (7014, 9684)	-694 (-1646, 445)	(-1236, -62)	0.002	0.039
	Placebo	73	8189 (6892, 9327)	-1187 (-2488, -250)	(-1711, -783)	<0.001	
OPG(CD19+) MFI	Calcium/Vit.D	63	30021 (24050, 40225)	-5890 (-9648, -164)	(-7250, -2715)	<0.001	0.71
	Placebo	73	29422 (23511, 42844)	-5474 (-12396, -1371)	(-6934, -3742)	<0.001	

[a] Wilcoxon signed rank p-value evaluating the within treatment group changes from baseline.

[b] Wilcoxon rank sum p-value evaluating the difference in changes from baseline between the two treatment groups

- Markers of CD4+ and CD8+ T cells and B cell activation decreased over 48 weeks in both treatment arms, that are characteristic of ART initiation.

- We also observed declines in level of OPG expression on CD4+ and CD8+ T cells and B cells in both treatment arms, but declines in RANKL expression were only observed on B cells (with VitD/Ca arm having smaller decline than the placebo arm).

- Greater declines in CD4+ T cell activation markers were modestly correlated with greater declines in lumbar spine and total hip BMD in both treatment arms. However, declines in CD8+ T cell activation and B cell activation correlated with declines in BMD in only one treatment arm (placebo or Ca/VitD arm, respectively; Table 3 & 4).

- Changes in senescence markers, CD4+ Tregs, or OPG and RANKL expression did not correlate with declines in BMD.

Table 3 and Figure 1: Spearman correlations between % change in total hip BMD and change in cellular markers from 0-48 weeks by treatment arm

Cellular Marker	Calcium/Vit.D N	r	P-value	Placebo N	r	P-value
% CD38+/HLA-DR+(CD4+)	61	0.25	0.047	72	0.26	0.026
% CD28-/CD57+(CD4+)	61	-0.09	0.49	72	-0.02	0.84
% Treg+(CD4+)	61	-0.00	0.98	72	0.18	0.13
% CD38+/HLA-DR+(CD8+)	61	0.07	0.59	72	0.31	0.009
% CD28-/CD57+(CD8+)	61	-0.20	0.11	72	0.07	0.57
% CD38+/HLA-DR+(CD19+)	60	0.30	0.020	71	0.11	0.38
RANKL+(CD4+) MFI	60	-0.05	0.70	71	0.15	0.21
OPG(CD4+) MFI	60	0.12	0.34	71	0.13	0.27
RANKL+(CD8+) MFI	60	-0.07	0.62	71	0.10	0.41
OPG(CD8+) MFI	60	0.13	0.31	71	0.10	0.38
RANKL+(CD19+) MFI	60	0.13	0.31	71	-0.01	0.92
OPG(CD19+) MFI	60	0.17	0.20	71	0.06	0.61
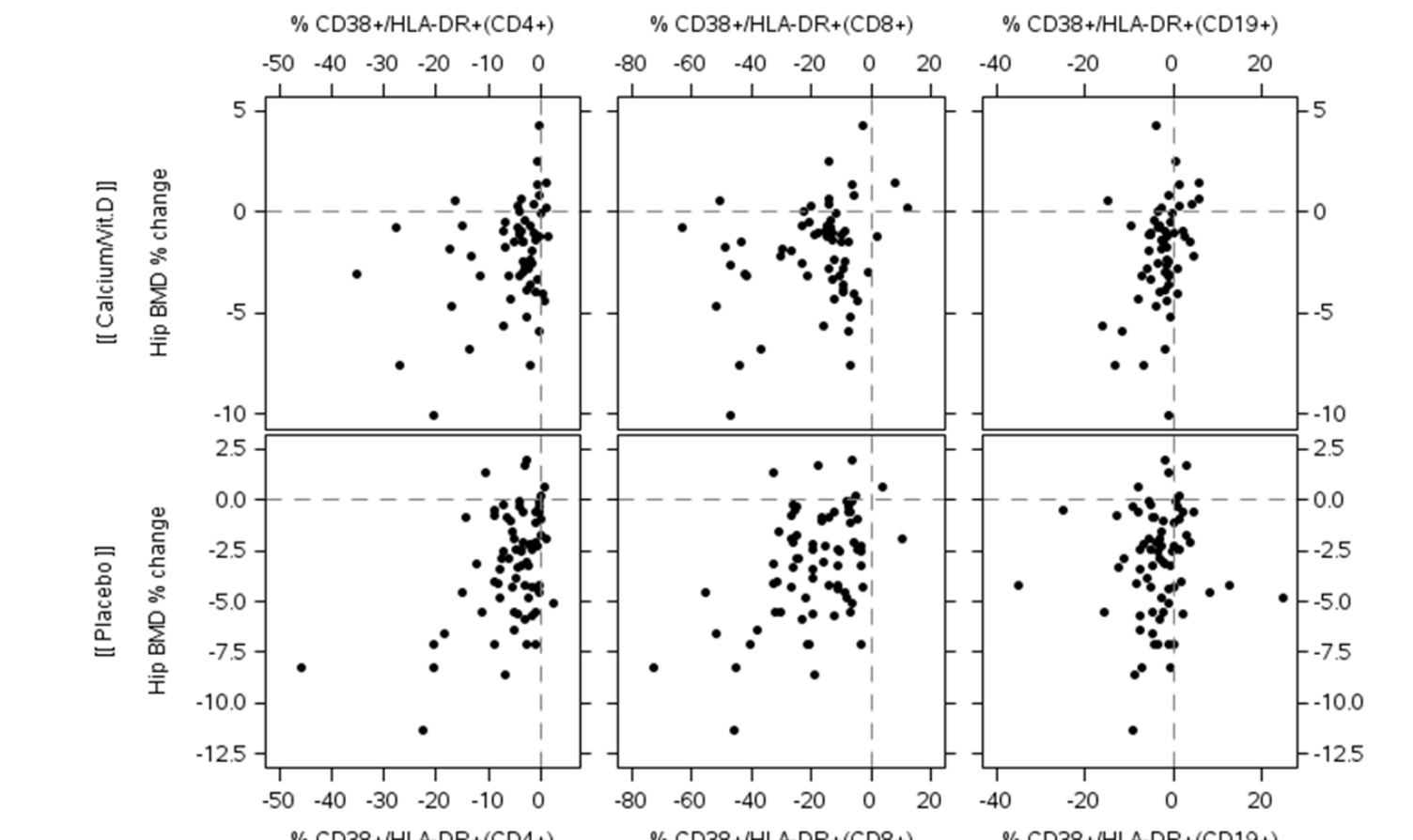
Table 4 and Figure 2: Spearman correlations between % change in lumbar spine BMD and change in cellular markers from 0-48 weeks by treatment arm

Cellular Marker	Calcium/Vit.D N	r	P-value	Placebo N	r	P-value
% CD38+/HLA-DR+(CD4+)	63	0.37	0.003	73	0.30	0.010
% CD28-/CD57+(CD4+)	63	0.07	0.60	73	0.08	0.51
% CD38+/HLA-DR+(CD8+)	63	0.20	0.12	73	0.25	0.035
% Treg+(CD4+)	63	0.18	0.17	73	0.14	0.22
% CD28-/CD57+(CD8+)	63	-0.02	0.88	73	0.16	0.17
% CD38+/HLA-DR+(CD19+)	63	0.22	0.09	73	0.22	0.22
RANKL+(CD4+) MFI	62	0.07	0.58	72	0.04	0.045
OPG(CD4+) MFI	62	0.07	0.62	72	0.02	0.89
RANKL+(CD8+) MFI	62	0.10	0.43	72	0.05	0.69
OPG(CD8+) MFI	62	-0.06	0.64	72	-0.01	0.90
RANKL+(CD19+) MFI	62	0.25	0.050	72	0.09	0.46
OPG(CD19+) MFI	62	0.07	0.58	72	0.00	1.00
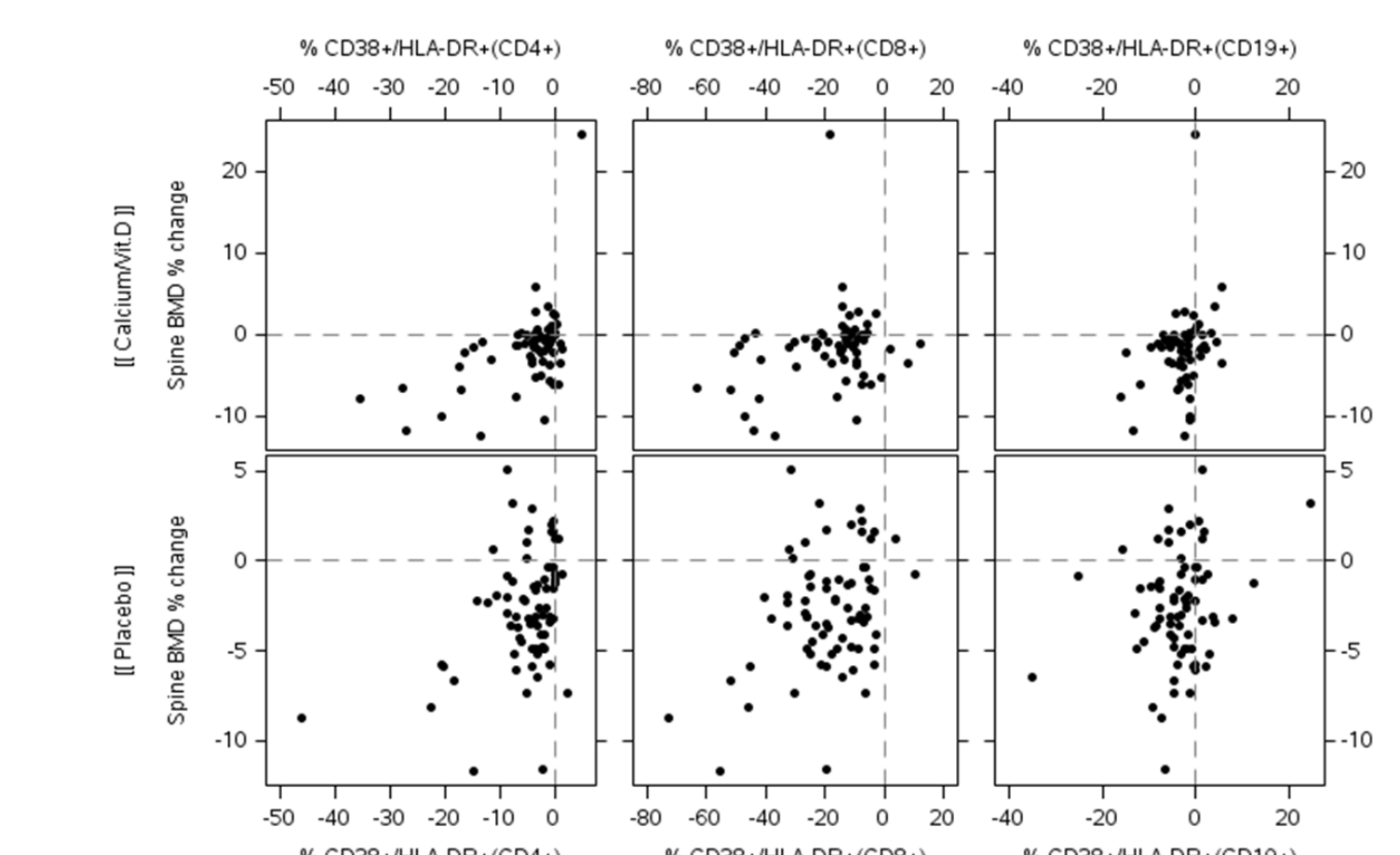
Conclusions

- Among the multiple cellular phenotypes analyzed in this exploratory analysis, no apparent differences between the VitD/Ca arm and placebo arm were observed, except that the VitD/Ca arm was found to have a lesser decline in RANKL expression on B cells than the placebo arm

- Reductions of T and B cell activation are characteristic of ART initiation. But VitD/Ca supplementation does not appear to augment the immunomodulatory effects of ART on T and B cells, or mitigate bone loss through modulation of immune activation.

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