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EFFECT OF HIGH DOSE VITAMIN D_3 ON THE HIV RESERVOIR: A RANDOMIZED CONTROLLED TRIAL

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In people living with HIV on suppressive ART, high dose vitamin D_3 treatment for 24 weeks was associated with no change in total HIV DNA frequency within CD4+ T cells at week 24 but ***a reduction in total HIV DNA frequency at week 36*** relative to placebo. This was accompanied by a ***reduction in frequency of effector memory CD4+ T cells*** and an ***increase in frequency of activated NK cells*** at week 36.

Background

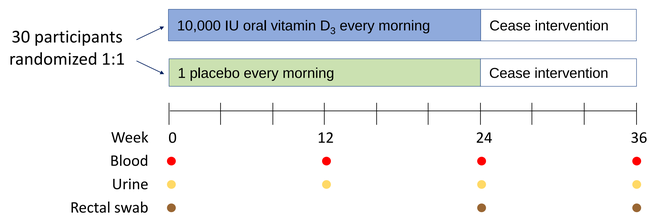
People living with HIV on suppressive ART have persistent gut and systemic inflammation. This may contribute to HIV persistence by promoting proliferation of latently infected CD4+ T cells and/or exhaustion of an effective immune response against HIV.

Vitamin D_3 is a steroid hormone with pleiotropic effects on the immune system and the gut. These include anti-proliferative effects on T cells,[1] reductions in CD8+ T cell activation and exhaustion[2] and improvements in gut barrier integrity[3] and dysbiosis.[4]

We hypothesized that high dose vitamin D_3 supplementation in people living with HIV on suppressive ART would reduce HIV persistence by reducing chronic inflammation directly through its effects on immune cells and indirectly through its effects on gut barrier integrity and microbiome diversity.

Methods

We performed a pilot randomized double-blind placebo-controlled trial at 3 trial sites within Melbourne, Australia. We included participants over 18 years of age living with HIV-1 on ART with plasma viral load suppressed below 40 copies/ml for at least 3 years. Participants were randomized 1:1 to receive 10,000 IU vitamin D_3 or placebo once a day for 24 weeks and were followed for a further 12 weeks. The primary endpoint was the difference between arms in the mean change in frequency of total HIV DNA within CD4+ T cells from week 0 to week 24. Secondary endpoints included a range of virologic and immunologic markers, pharmacokinetics and safety. All analyses were by intention to treat with no imputation made for missing data. Virologic endpoints were analyzed using mixed effects negative binomial regression models and immunologic and 25-hydroxyvitamin D endpoints with mixed effects linear models.



Results: 1. Participants

Of 42 potential participants screened, 30 were enrolled and randomized,15 to each arm.

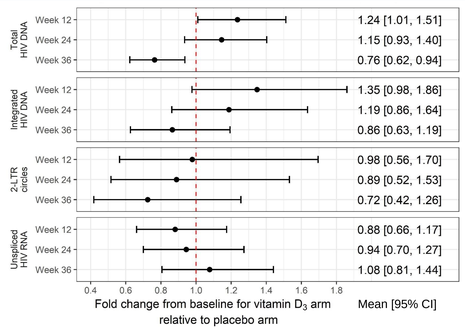
3 participants in the vitamin D_3 arm and 2 in the placebo arm withdrew prior to the primary endpoint.

Baseline demographics	Placebo (n = 15)	Vitamin D_3 (n = 15)	Total (N = 30)
Age, median (IQR), years	49 (38-54)	50 (35-52)	49 (38-52)
Gender – Cisgender male, no. (%)	15 (100%)	15 (100%)	30 (100%)
Race, no. (%)			
•Indigenous Australian, no. (%)	0 (0%)	1 (7%)	1 (3%)
•White, no. (%)	14 (93%)	13 (87%)	27 (90%)
•Black, no. (%)	1 (7%)	0 (0%)	1 (3%)
•Pacific Islander, no. (%)	0 (0%)	1 (7%)	1 (3%)
Duration of HIV, median (IQR), years	12 (9-15)	11 (6-18)	11 (7-16)
CD4 nadir, median (IQR), cells/μL	320 (180-476)	265 (52-499)	280 (109-484)
Time since CD4 nadir, median (IQR), yrs	9 (4-12)	7 (5-11)	8 (5-12)
Most recent CD4, median (IQR), cells/μL	700 (630-1154)	830 (701-910)	782 (635-1052)

All withdrawals were for personal reasons apart from one placebo arm participant who withdrew from study drug after week 12 due to an adverse event (grade 2 constipation) but remained on the study and completed all study procedures as per protocol.

Thus 12 participants in the vitamin D_3 arm and 14 in the placebo arm were evaluated for the primary endpoint. We present final results of the completed study.

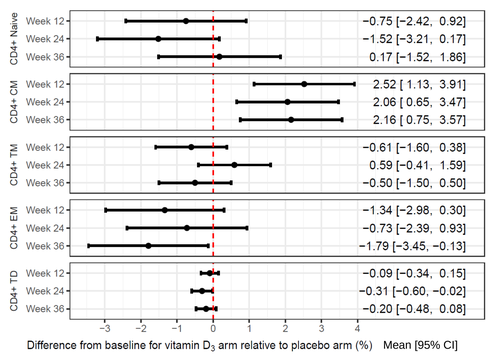
Results: 2. Virology



Vitamin D_3 was associated with no change in frequency of total HIV DNA at week 24 but there was an increase at week 12 and a decrease at week 36 in frequency of total HIV DNA in the vitamin D_3 arm relative to the placebo arm.

Integrated HIV DNA moved in the same direction as total HIV DNA at each time point but was not significant. There were no significant findings for 2-LTR circles or unspliced HIV RNA at any time point.

Results: 3. CD4+ T cell maturation



There was an overall shift away from the more differentiated effector memory and terminally differentiated CD4+ T cell subsets towards the less differentiated central memory CD4+ T cell subset in the vitamin D_3 arm relative to placebo. This is in keeping with the known anti-proliferative effect of vitamin D_3 on CD4+ T cells.[5] Similar findings were seen for CD8+ T cells (data not shown).

CM = central memory, TM = transitional memory, EM = effector memory, TD = terminally differentiated

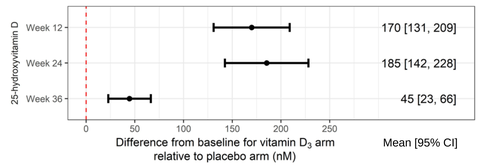
Results: 4. Other immunology

Parameter	Parent	Week 12 vs baseline (n = 27)	Week 24 vs baseline (n = 26)	Week 36 vs baseline (n = 26)
CCR6+ (%)	CD4+ T cells	1.3 [-0.1, 2.7]	2.0 [0.6, 3.4]	0.9 [-0.6, 2.3]
CCR6+CXCR3- (%)	CD4+ T cells	1.0 [0.2, 1.9]	1.1 [0.2, 1.9]	0.6 [-0.2, 1.5]
CCR6-CXCR3+ (%)	CD4+ T cells	-2.5 [-4.1, -1.0]	0.0 [-1.6, 1.6]	-1.0 [-2.6, 0.5]
CD38+HLA-DR+ (%)	CD8+ T cells	0.98 [0.32, 1.65]	0.28 [-0.39, 0.96]	0.51 [-0.16, 1.19]
PD-1+ (%)	CD8+ T cells	1.0 [-1.1, 3.1]	3.1 [0.9, 5.2]	1.0 [-1.1, 3.1]
CD38+HLA-DR+ (%)	NK cells	0.17 [-0.13, 0.48]	0.40 [0.07, 0.73]	0.43 [0.08, 0.77]
NKG2A+NKG2C- (%)	NK cells	1.9 [0.4, 3.5]	1.9 [0.4, 3.5]	1.4 [-0.2, 2.9]
NKG2A-NKG2C+ (%)	NK cells	-2.2 [-4.4, -0.1]	-2.5 [-4.6, -0.3]	-1.4 [-3.6, 0.7]

Increase Decrease Mean [95% CI]

There were unexpected increases in Th17 (CCR6+ CD4+) cell, activated and exhausted CD8+ T cell and activated NK cell frequencies relative to placebo. Th1 (CXCR3+ CD4+) cell frequency decreased as expected. There was a shift away from NKG2C+ towards NKG2A+ NK cells which are known to be able to kill HIV-infected CD4+ T cells potently.[6]

Results: 5. Pharmacokinetics and safety



25-hydroxyvitamin D rose in the vitamin D_3 arm at weeks 12 and 24 before falling at week 36 but remained elevated at week 36 relative to placebo consistent with its known long half-life in vivo.[7]

No significant safety issues were identified throughout the trial.

Conclusions

Virology: No effect of high dose vitamin D_3 was seen on total HIV DNA within CD4+ T cells at week 24 but a decrease in total HIV DNA was observed at week 36 relative to placebo.

Immunology: A shift towards less mature CD4+ T cells, shifts from Th1 to Th17 cells and from NKG2C+ to NKG2A+ NK cells and increased activation of CD8+ T cells and NK cells were seen relative to placebo.

Pharmacokinetics and safety: High 25-hydroxyvitamin D levels were achieved at weeks 12 and 24 without safety issues and levels remained elevated at week 36 in the vitamin D_3 arm relative to placebo.

Possible explanations for a decrease in HIV DNA at week 36 include

- reduced frequency of effector memory CD4+ T cells which are known to be enriched in HIV DNA.
- increased frequency of activated NK cells and CD8+ T cells which could potentially kill HIV-infected CD4+ T cells.
- Prolonged exposure to vitamin D_3 might be needed given that these findings were seen in the context of persistently elevated levels of 25-hydroxyvitamin D.

Finally we acknowledge that this was a small study and that the effect size on HIV DNA at week 36 was small; further studies are therefore indicated.

In association with

References

1. Burton et al 2010 *Neurology* 74: 1852-9; 2. Pincikova et al 2017 *Clin Exp Immunol* 189: 359-71; 3. Liu et al 2013 *J Clin Invest* 123: 3983-96; 4. Bashir et al 2016 *Eur J Nutr* 55: 1479-89; 5. Datta-Mitra et al 2013 *Int Immunopharmacol* 17: 744-51; 6. Davis et al 2016 *PLoS Pathog* 12: e1005421; 7. Jones et al 2014 *J Clin Endocrinol Metab* 99: 3373-81

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