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Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Sudfeld CR, Mugusi F, Muhihi A, et al. Efficacy of vitamin D_3 supplementation for the prevention of pulmonary tuberculosis and mortality in HIV: a randomised, double-blind, placebo-controlled trial. *Lancet HIV* 2020; **7:** e463–71.

Supplementary Material for

Efficacy of vitamin D supplementation for the prevention of pulmonary tuberculosis and mortality in HIV: a randomised, double-blind, placebo-controlled trial

Sudfeld, et al.

Secondary outcomes included in trial protocol to be reported in the second half of 2020

(i) post-randomization CD4 T cell count as mean difference in cells/μL,

(ii) incidence of comorbidities and complications associated with HIV and/or ART,

(iii) post-randomization body mass index (BMI) as mean difference in kg/m2,

(iv) incidence of wasting (BMI <18.5 kg/m2) and >10% weight loss from baseline,

(v) post-randomization depression and anxiety as mean difference in Hopkins Symptom Checklist (HSCL) scores,

(vi) post-randomization physical activity as mean difference in metabolic equivalents (METs),

(vii) post-randomization bone health biomarkers [parathyroid hormone (PTH) and alkaline phosphatase (ALP)] as mean difference in concentrations

(viii) post-randomization immunologic biomarkers [interlukin-2 (IL-2), interlukin-12 (IL-12), interferon-gamma (IFN-γ), and the antimicrobial peptide cathelicidin as mean difference in concentrations

Supplemental Table 1. Mean serum 25(OH)D ng/mL, percentage of participants with 25 (OH)D <30 ng/mL, and mean difference in 25(OH)D at baseline (n=399), 1 month (n=313), 6 months (n=357), and 12 months (n=314) of follow-up

	Baseline Mean ± SD	Baseline % <30 ng/mL / % <20 ng/mL	Month 1 Mean ± SD 25(OH)D ng/mL	Month 1 % <30 ng/mL / % <20 ng/mL	Month 6 Mean ± SD	Month 6 % <30 ng/mL / % <20 ng/mL	Month 12 Mean ± SD	Month 12 % <30 ng/mL / % <20 ng/mL
Vitamin D (n=207)	27.9 ± 7.7	63.8% / 16.4%	50.7 ± 12.8	3.0% / 0.0%	38.0 ± 8.4	13.8% / 1.6%	38.1 ± 8.5	12.3% / 1.2%
Placebo (n=192)	27.3 ± 7.9	71.4% / 14.6%	27.8 ± 7.0	62.2% / 10.8%	29.3 ± 7.4	56.6% / 11.9%	27.6 ± 7.2	62.9% / 14.6%
Mean difference in 25(OH)D concentration ng/mL for vitamin D compared to placebo (95% CI)	0.6 (-1.1, 2.2)		23.0 (21.1, 24.8)		8.7 (7.0, 10.5)		10.4 (8.6, 12.3)	
p-value for mean difference	0.52		<0.0001		<0.0001		<0.0001	

Supplemental Table 2. Distribution of cause of death by randomized treatment arm (n=415 deaths)

	Vitamin D n=211 n (%)	Placebo n=204 n (%)
Pulmonary TB	42 (19.9)	43 (21.1)
Extrapulmonary TB	3 (1.4)	3 (1.5)
Infection, non-specific	40 (19.0)	36 (17.7)
Diarrhea	19 (9.0)	28 (13.7)
Cardiovascular disease / stroke	26 (12.3)	16 (7.8)
Cryptococcal meningitis	11 (5.2)	10 (4.9)
Pneumonia	9 (4.3)	10 (4.9)
Anemia	4 (1.9)	8 (3.9)
Kidney failure	6 (2.8)	4 (2.0)
Meningitis/encephalitis	6 (2.8)	3 (1.5)
Cancers	4 (1.9)	4 (2.0)
Liver failure	2 (1.0)	5 (2.5)
Pneumocystis pneumonia	4 (1.9)	2 (1.0)
Malaria	4 (1.9)	1 (0.5)
Kaposi's sarcoma	1 (0.5)	1 (0.5)
Motor vehicle accident	2 (1.0)	0 (0.0)
Other*	7 (3.3)	2 (1.0)
Unknown	21 (10.0)	28 (13.7)

*Other includes starvation (5), dehydration (1), intestinal obstruction (1), gastrointestinal hemorrhage (1) and pulmonary embolism (1).

Supplemental Table 3. Baseline demographic and health characteristics of randomized participants with p-values for differences to inform sensitivity analyses accounting for potential imbalances (n=4,000)

	Vitamin D N=2,001 n (%) or mean ± SD	Placebo N=1,999 n (%) or mean ± SD	p-value
Female	1367 (68.3)	1368 (68.4)	0.94
Mean age, years	38.6 ± 9.8	38.8 ± 10.0	0.64
Education			
No formal education	308 (15.4)	327 (16.4)	0.77
Primary	1294 (64.7)	1294 (64.7)	
Secondary/ advanced	398 (19.9)	377 (18.9)	
Body mass index (kg/m2)			
<18.5 kg/m^2	440 (22.0)	404 (20.2)	0.27
18.5-24.9 kg/m^2	1038 (51.9)	1064 (53.2)	
≥ 25.0 kg/m^2	521(26.0)	531 (26.6)	
CD4 T-cell count			
< 200 cells per µL	866 (43.3)	845 (42.3)	0.62
200-349 cells per µL	461 (23.0)	445 (22.3)	
350-499 cells per µL	300 (15.0)	333 (16.7)	
≥ 500 cells per µL	284 (14.2)	278 (13.9)	
Missing	90 (4.5)	98 (4.9)	
WHO HIV disease stage			
I / II	744 (37.2)	760 (38.0)	0.86
III	1161 (58.0)	1143 (57.2)	
IV	96 (4.8)	96 (4.8)	
Baseline pulmonary TB	189 (9.5)	175 (8.8)	0.45
Isoniazid preventive therapy	35 (1.8)	27 (1.4)	0.31
HAART regimen			
Efavirenz/lamivudine/tenofovir (EFV/3TC/TDF)	1940 (97.0)	1943 (97.2)	0.94
Other HAART regimen	61 (3.0)	56 (2.8)	
Vitamin D status at screening visit			
Insufficient 25(OH)D 20.0- 30.0 ng/mL	955 (47.7)	972 (48.6)	0.042
Moderate deficiency 25(OH)D 10.0- 19.9 ng/mL	920 (46.0)	865 (43.3)	
Severe deficiency 25(OH)D 0-9.9 ng/mL	126 (6.3)	162 (8.1)	

Supplemental Table 4. Sensitivity analysis of the effect of vitamin D_3 supplementation on all-cause mortality and incident pulmonary tuberculosis adjusting for baseline vitamin D level and CD4 T-cell count

	Vitamin D No. events / No. randomized at risk (%)	Placebo No. events / No. randomized at risk (%)	Adjusted* Hazard ratio (95% CI)	P value
All-cause mortality	211 / 2001 (10.5)	204 / 1999 (10.2)	1.02 (0.84-1.24)	0.85
Incident pulmonary TB	50 / 1812 (2.8)	64 / 1827 (3.5)	0.78 (0.54-1.13)	0.18
Incident sputum smear-positive pulmonary TB	20 / 1812 (1.1)	37 / 1827 (2.0)	0.53 (0.31-0.92)	0.026
Incident sputum smear-negative pulmonary TB	30 / 1812 (1.6)	27 / 1827 (1.5)	1.12 (0.66-1.88)	0.68
Incident microbiologically confirmed TB (sputum or GeneXpert positive)	22 / 1812 (1.2)	38 / 1827 (2.1)	0.58 (0.34-0.97)	0.39
Incident high bacillary load (≥ 2+) sputum smear-positive pulmonary TB	10 / 1812 (0.6)	27 / 1827 (1.5)	0.37 (0.18-0.76)	0.0065

*Adjusted for baseline vitamin D concentration (<10, 10-19.9, ≥20 ng/mL) and CD4 T-cell count (missing, <200, 200-349, 350-499, ≥ 500, cells per μL)

Supplemental Table 5. Sensitivity analysis of the analysis of effect modification of vitamin D_3 on mortality by pre-specified baseline factors adjusting for baseline vitamin D level and CD4 T-cell count

	Vitamin D n / N (%)	Placebo n / N (%)	Hazard Ratio (95% CI)	P-value for effect modification
All participants	211 / 2001 (10.5)	204 / 1999 (10.2)	1.04 (0.85-1.25)	-
Subgroups				
Sex				
Male	105 / 634 (16.6)	93 / 631 (14.7)	1.15 (0.87-1.52)	0.29
Female	106 / 1367 (7.8)	111 /1368 (8.1)	0.93 (0.71-1.22)	
Age				
< 40 years	101 / 1176 (8.6)	90 / 1130 (8.0)	1.05 (0.80-1.40)	0.90
≥ 40 years	110 / 825 (13.3)	114 / 755 (13.1)	1.03 (0.79-1.34)	
CD4 T-cell count				
< 200 cells per μL	134 / 866 (15.5)	141 / 845 (16.7)	0.91 (0.72-1.16)	0.17
≥ 200 cells per μL	60 / 1045 (5.7)	44 / 1056 (4.2)	1.41 (0.96-2.08)	
Missing	17 / 90 (18.9)	19 / 98 (19.4)	0.98 (0.51-1.90)	
WHO HIV disease stage				
I / II	29 / 744 (3.9)	38 / 760 (5.0)	0.80 (0.49-1.29)	0.013
III	160 / 1161 (13.8)	128 / 1143 (11.2)	1.20 (0.95-1.51)	
IV	22 / 96 (22.9)	38 / 96 (39.6)	0.54 (0.32-0.92)	
Baseline pulmonary TB				
Yes	23 / 189 (12.2)	35 / 175 (20.0)	0.58 (0.34-0.99)	0.023
No	188 / 1812 (10.4)	169 / 1824 (9.3)	1.11 (0.90-1.37)	
Body mass index				
<18.5 kg/m^2	94 / 440 (21.4)	83 / 404 (20.5)	0.98 (0.73-1.32)	0.99
≥ 18.5 kg/m^2	115 / 1559 (7.4)	121 / 1595 (7.6)	0.98 (0.76-1.25)	
Vitamin D status at screening visit				
Deficient 25(OH)D 0-19.9 ng/mL	104 / 1046 (9.9)	98 / 1027 (9.5)	1.03 (0.78-1.36)	0.97
Insufficient 25(OH)D 20.0-29.9 ng/mL	107 / 955 (11.2)	106 / 972 (10.9)	1.02 (0.78-1.33)	
Adherence to randomized trial regimen				
High – 100% on weekly doses and ≥90% on daily doses	103 / 876 (11.8)	110 / 889 (12.4)	0.92 (0.70-1.22)	0.28
Low – <100% on weekly doses or < 90% on daily doses	108 / 1125 (9.6)	94 / 1110 (8.5)	1.14 (0.87-1.50)	

*Adjusted for baseline vitamin D level (<10, 10-19.9, ≥20 ng/mL) and CD4 T-cell count (missing, <200, 200-349, 350-499, ≥ 500 cells per μL)

Supplemental Table 6. Modifiers of the effect of vitamin D_3 on incidence of pulmonary tuberculosis

	Vitamin D n / N (%)	Placebo n / N (%)	Hazard Ratio (95% CI)	P-value for effect modification
All participants	50 / 1812 (2.8)	64 / 1827 (3.5)	0.78 (0.54-1.13)	-
Subgroups				
Sex				
Male	27 / 535 (5.1)	26 / 534 (4.9)	1.03 (0.61-1.78)	0.15
Female	23 / 1277 (1.8)	38 / 1293 (3.0)	0.60 (0.36-1.02)	
CD4 T-cell count				
<200 cells per μL	31 / 753 (4.1)	34 / 741 (4.6)	0.87 (0.54-1.42)	0.74
≥200 cells per μL	18 / 983 (1.8)	28 / 998 (2.8)	0.66 (0.36-1.19)	
WHO HIV disease stage				
I / II	14 / 741 (1.9)	19 / 753 (2.5)	0.74 (0.37-1.48)	0.25
III	32 / 994 (2.7)	44 / 994 (4.4)	0.72 (0.46-1.14)	
IV	4 / 79 (5.1)	1 / 79 (1.3)	3.87 (0.44-34.1)	
Baseline vitamin D status				
Insufficient 25(OH)D ≥20 ng/mL and <30 ng/mL	23 / 855 (2.7)	34 / 874 (3.9)	0.68 (0.40-1.15)	0.50
Deficient 25(OH)D <20 ng/mL	27 / 957 (2.8)	30 / 953 (3.2)	0.90 (0.54-1.51)	
Adherence to trial regimen				
High – 100% on weekly doses and ≥90% on daily doses	20 / 803 (2.5)	22 / 811 (2.7)	0.91 (0.50-1.67)	0.53
Low – <100% on weekly doses or <90% on daily doses	30 / 1009 (3.0)	42 / 1013 (4.2)	0.71 (0.45-1.14)	

Supplemental Table 7. Sensitivity analysis of the analysis of effect modification of vitamin D_3 on incidence of pulmonary tuberculosis by pre-specified baseline factors adjusting for baseline vitamin D level and CD4 T-cell count

	Vitamin D n / N (%)	Placebo n / N (%)	Hazard Ratio (95% CI)	P-value for effect modification
Sex				
Male	27 / 535 (5.1)	26 / 534 (4.9)	1.07 (0.62-1.83)	0.12
Female	23 / 1277 (1.8)	38 / 1293 (3.0)	0.60 (0.35-1.00)	
CD4 T-cell count				
<200 cells per μL	31 / 753 (4.1)	34 / 741 (4.6)	0.88 (0.54-1.43)	0.74
≥200 cells per μL	18 / 983 (1.8)	28 / 998 (2.8)	0.67 (0.37-1.20)	
WHO HIV disease stage				
I / II	14 / 741 (1.9)	19 / 753 (2.5)	0.75 (0.37-1.49)	0.44
III	32 / 994 (2.7)	44 / 994 (4.4)	0.72 (0.45-1.13)	
IV	4 / 79 (5.1)	1 / 79 (1.3)	3.80 (0.45-32.1)	
Baseline vitamin D status				
Insufficient 25(OH)D ≥20 ng/mL and <30 ng/mL	23 / 855 (2.7)	34 / 874 (3.9)	0.67 (0.40-1.15)	0.50
Deficient 25(OH)D <20 ng/mL	27 / 957 (2.8)	30 / 953 (3.2)	0.87 (0.52-1.46)	
Adherence to trial regimen				
High – 100% on weekly doses and ≥90% on daily doses	20 / 803 (2.5)	22 / 811 (2.7)	0.90 (0.48-1.62)	0.60
Low – <100% on weekly doses or <90% on daily doses	30 / 1009 (3.0)	42 / 1013 (4.2)	0.72 (0.45-1.16)	

*Adjusted for baseline vitamin D level (<10, 10-19.9, ≥20 ng/mL) and CD4 T-cell count (missing, <200, 200-349, 350-499, ≥ 500 cells per μL)

Supplemental Table 8. Sensitivity analysis of the effect of vitamin D_3 supplementation on pulmonary TB incidence excluding cases in the first 14, 30, and 90 days of follow-up.

	Vitamin D No. events / No. randomized at risk (%)	Placebo No. events / No. randomized at risk (%)	Hazard ratio (95% CI)
Incident pulmonary TB excluding cases ≤ 14 days	45 / 1772 (2.5)	52 / 1772 (2.9)	0.87 (0.58-1.29)
Incident pulmonary TB excluding cases ≤ 30 days	32 / 1717 (1.9)	38 / 1727 (2.2)	0.84 (0.53-1.35)
Incident pulmonary TB excluding cases ≤ 90 days	17 / 1634 (1.0)	24 / 1653 (1.5)	0.71 (0.38-1.32)
Incident sputum smear-positive pulmonary TB excluding cases ≤ 14 days	17 / 1772 (1.0)	30 / 1772 (1.7)	0.56 (0.31-1.02)
Incident sputum smear-positive pulmonary TB excluding cases ≤ 30 days	10 / 1717 (0.6)	21 / 1727 (1.2)	0.47 (0.22-1.00)
Incident sputum smear-positive pulmonary TB excluding cases ≤ 90 days	1 / 1634 (0.1)	13 / 1653 (0.8)	0.08 (0.01-0.58)
Incident sputum smear-negative pulmonary TB excluding cases ≤ 14 days	28 / 1772 (1.6)	22 / 1772 (1.2)	1.28 (0.73-2.24)
Incident sputum smear-negative pulmonary TB excluding cases ≤ 30 days	22 / 1717 (1.3)	17 / 1727 (1.0)	1.30 (0.69-2.46)
Incident sputum smear-negative pulmonary TB excluding cases ≤ 90 days	16 / 1634 (1.0)	11 / 1653 (0.7)	1.46 (0.68-3.15)

Supplemental Table 9. Adverse events by randomized treatment arm

	Vitamin D	Placebo
Hypercalcemia (Grade 1)	3	2
Total hospitalizations (Grade 3)	101	94
Reason for hospitalization		
Suspected infection	19	25
Malaria	17	15
Anemia	14	21
Diarrhea	10	1
Pneumonia	5	4
Vomiting	5	2
Cardiovascular-related	5	5
Dehydration	4	4
Tuberculosis	3	3
Kaposi's sarcoma	3	0
Injury	3	4
Peripheral edema	3	1
Post-operative complications	2	3
Abdominal pain	2	4
Mental illness	1	0
Cancer	1	0
Kidney failure	1	0
Uterine fibroids	1	0
Lower limb pain	1	0
Peptic ulcer	1	0
Loss of consciousness	0	2