High-dose vitamin D_3 in adults with pulmonary tuberculosis: a double-blind randomized controlled trial[1,2]

*Nestan Tukvadze,[3] Ekaterina Sanikidze,[3] Maia Kipiani,[3] Gautam Hebbar,[4] Kirk A Easley,[7] Neeta Shenvi,[7] Russell R Kempker,[4,5] Jennifer K Frediani,[4,6] Veriko Mirtskhulava,[3,4] Jessica A Alvarez,[4,6] Nino Lomtadze,[3] Lamara Vashakidze,[3] Li Hao,[4] Carlos Del Rio,[5,9] Vin Tangpricha,[4,6,10] Henry M Blumberg,[5,8] and Thomas R Ziegler[4,6]**

[3]National Center for Tuberculosis and Lung Disease, Tbilisi, Georgia; Divisions of [4]Endocrinology, Metabolism and Lipids and [5]Infectious Diseases and [6]Center for Clinical and Molecular Nutrition, Department of Medicine, Emory University School of Medicine, Atlanta, GA; Departments of [7]Biostatistics and Bioinformatics and [8]Epidemiology and [9]Hubert Department of Global Health, Rollins School of Public Health, Emory University, Atlanta, GA; and [10]Atlanta Veterans Affairs Medical Center, Decatur, GA

ABSTRACT

Background: Tuberculosis, including multidrug-resistant tuberculosis (MDR-TB), is a major global health problem. Individuals with tuberculosis disease commonly exhibit vitamin D deficiency, which may adversely affect immunity and the response to therapy.

Objective: We determined whether adjunctive high-dose vitamin D_3 supplementation improves outcomes in individuals with pulmonary tuberculosis disease.

Design: The study was a double-blind, randomized, placebo-controlled, intent-to-treat trial in 199 individuals with pulmonary tuberculosis disease in Tbilisi, Georgia. Subjects were randomly assigned to receive oral vitamin D_3 [50,000 IUs (1.25 mg) thrice weekly for 8 wk and 50,000 IU every other week for 8 wk] or a placebo concomitant with standard first-line antituberculosis drugs. The primary outcome was the time for the conversion of a *Mycobacterium tuberculosis* (*Mtb*) sputum culture to negative.

Results: Baseline characteristics between groups were similar. Most subjects (74%) were vitamin D deficient (plasma 25-hydroxyvitamin D [25(OH)D] concentration $<$50 nmol/L). With vitamin D_3, plasma 25(OH)D concentrations peaked at $\sim$250 nmol/L by 8 wk and decreased to $\sim$125 nmol/L at week 16. Adverse events and plasma calcium concentrations were similar between groups. In 192 subjects with culture-confirmed tuberculosis, an adjusted efficacy analysis showed similar median culture-conversion times between vitamin D_3 and placebo groups [29 and 27 d, respectively; HR: 0.86; 95% CI: 0.63, 1.18; P = 0.33). Eight-week culture-conversion rates were also similar (84.0% and 82.1% for vitamin D_3 and placebo, respectively; P = 0.99).

Conclusion: A high-dose vitamin D_3 regimen safely corrected vitamin D deficiency but did not improve the rate of sputum *Mtb* clearance over 16 wk in this pulmonary tuberculosis cohort. This trial was registered at clinicaltrials.gov at NCT00918086. *Am J Clin Nutr* 2015;102:1059–69.

Keywords: multidrug-resistant tuberculosis, *Mycobacterium tuberculosis*, randomized controlled trial, tuberculosis, vitamin D

INTRODUCTION

Tuberculosis is a major global public health problem (1–4). The WHO estimated that, in 2013, there were a total of 9.0 million new tuberculosis cases worldwide and 1.5 million deaths that were attributable to tuberculosis (1). The same year, the WHO also estimated that 480,000 people developed multidrug-resistant tuberculosis (MDR-TB),[11] which was responsible for $\sim$170,000 deaths worldwide (1). Because of these challenges, the 2014 WHO Global Tuberculosis Report strongly advocated for new tuberculosis medicines to control the global tuberculosis epidemic (1).

Current evidence suggests a link between vitamin D and a variety of immune functions (5–10). Vitamin D deficiency is common in active tuberculosis (11–14). Vitamin D deficiency has also been associated with a susceptibility to tuberculosis infection, the conversion from latent to active tuberculosis, and the severity and relapse rate of tuberculosis disease (11–16). Studies have shown that the 1,25-dihydroxyvitamin D_3 induction of antimycobacterial activity in monocytes is mediated in part by the upregulation of the antimicrobial peptide LL-37 (5–9).

Because of the pleiotropic effects of vitamin D on immunity, several randomized clinical trials (RCTs) of vitamin D supplementation in patients with pulmonary tuberculosis disease have been performed (17–23). Unfortunately, the efficacy of adjunctive

[1] Supported in part by NIH grants UL1 TR000454 (to the Atlanta Clinical and Translational Science Institute), K24 DK096574 (to TRZ), D43 TW007124 (to HMB), D43 TW001042 (to CDR), K23 AR054334 (to VT), K23 AI103044 (to RRK), and T32 DK007298-32S1 and K01 DK102851 (to JAA) and the Emory University Global Health Institute (to TRZ, VT, HMB, CD, and RRK).

[2] Supplemental Material and Supplemental Tables 1–4 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

*To whom correspondence should be addressed. E-mail: tzieg01@emory.edu.

[11] Abbreviations used: AFB, acid-fast bacilli; CRF, case report form; MDR-TB, multidrug-resistant tuberculosis; *Mtb*, *Mycobacterium tuberculosis*; NCTLD, National Center for Tuberculosis and Lung Disease; RCT, randomized clinical trial; VDR, vitamin D receptor; VitDQAP, Vitamin D Metabolites QA Program; 25(OH)D, 25-hydroxyvitamin D.

Received April 30, 2015. Accepted for publication August 20, 2015.

First published online September 23, 2015; doi: 10.3945/ajcn.115.113886.

vitamin D may be difficult to interpret because of various aspects of study designs, the endpoints chosen, missing data, a lack of measurement of 25-hydroxyvitamin D [25(OH)D] (21), and varying doses of vitamin D (18–23), which, in some studies, failed to increase blood 25(OH)D concentrations (18). In the current study, we tested the hypothesis that adjunctive high-dose vitamin D_3 administration, which was designed to safely increase plasma 25(OH)D concentrations into a sustained supraphysiologic range, would significantly enhance the clearance of *Mycobacterium tuberculosis* (*Mtb*) from sputum over time in adults with newly diagnosed pulmonary tuberculosis disease in Tbilisi, Georgia.

METHODS

Informed consent

The Institutional Review Board of Emory University and the Georgian National Center for Tuberculosis and Lung Disease (NCTLD) Ethics Committee approved the study (clinicaltrials.gov; NCT00918086).

Study subjects

Patients with pulmonary tuberculosis disease were recruited between July 2009 and April 2012 from the Georgian NCTLD and an affiliated tuberculosis clinic in Tbilisi, Georgia. Medical records of consecutive patients with newly diagnosed pulmonary tuberculosis disease were screened for eligibility. Potential study subjects were identified after diagnosis with the use of standard methods of the NCTLD [compatible signs and symptoms, a positive sputum acid-fast bacilli (AFB) smear microscopy, and a chest radiograph]. Informed consent was obtained from all subjects.

Inclusion criteria were as follows: *1*) age $\geq$18 y, *2*) newly diagnosed pulmonary tuberculosis disease as determined by symptoms and signs of a positive AFB sputum smear (later confirmed by a positive sputum culture for *Mtb* for a modified intent-to-treat analysis), 3) $\leq$7 d of antituberculosis drug therapy before entry, and *4*) the ability to provide informed consent. Exclusion criteria were as follows: *1*) a previous diagnosis of tuberculosis disease; *2*) current extrapulmonary tuberculosis; *3*) a pregnancy or lactating mother; *4*) a history of hypercalcemia, nephrolithiasis, hyperparathyroidism, sarcoidosis, organ transplant, hepatic cirrhosis, seizures, or cancer in the past 5 y; *5*) a baseline plasma calcium concentration $>$2.6 mmol/L, creatinine concentration $>$250 μmol/L, or aspartate aminotransferase concentrations that was $>$3 times the upper limit of the normal range; *6*) a requirement for renal replacement therapy; *7*); corticosteroid use in the past 30 d; *8*) current use of cytotoxic or immunosuppressive drugs; *9*) known MDR-TB before study enrollment; and *10*) current incarceration.

Sputum smear and culture and drug-susceptibility testing

Duplicate sputum specimens were collected for an AFB smear and culture at baseline and at weeks 2, 4, 6, 8, 12, and 16. A smear microscopy was performed at the NCTLD by the National Tuberculosis Reference Laboratory with the use of Ziehl-Neelsen staining as previously described (24, 25). All sputum samples had AFB cultures performed with the use of Löwenstein-Jensen solid media and standard methodologies as previously reported (24, 25). Positive cultures were confirmed to be *Mtb* complex with the use of phenotypic tests and the Genotype MTBDR*plus* assay (Hain Lifescience; www.hain-lifescience.de) (24, 25).

Drug-susceptibility testing was performed with the use of the absolute concentration method on solid media as previously described (25). In 2011, the Georgian National Tuberculosis Reference Laboratory implemented the line probe assay (MTBDR*plus*) for use on all AFB smear-positive sputum samples for the detection of MDR-TB (25). MDR-TB was defined as resistance to at least isoniazid and rifampicin by either drug-susceptibility testing or a line probe assay (1, 2).

Antituberculosis drug therapy

Antituberculosis drug therapy (isoniazid, rifampicin, pyrazinamide, and ethambutol) was started in all subjects no sooner than 7 d from entry on the basis of WHO recommendations (4, 23, 24). Subjects with confirmed MDR-TB were changed to appropriate second-line drug therapy per standard clinical care protocols of the NCTLD. All subjects enrolled into the trial received concomitant directly observed therapy of antituberculosis drugs and the study drug (24, 25).

Baseline blood chemistries and serial calcium and 25(OH)D concentrations

Plasma was obtained at baseline for concentrations of calcium, creatinine, and aspartate aminotransferase. Plasma calcium concentrations were serially monitored in all subjects at weeks 4, 8, and 12 and, if the subject had suggestive symptoms of hypercalcemia, also at weeks 2, 6, and 16.

Plasma was obtained at baseline and weeks 2, 4, 8, 12, and 16 for measurements of 25-hydroxyvitamin D_2 and 25-hydroxyvitamin D_3 concentrations, respectively, in one single batch with the use of liquid chromatography–tandem mass spectrometry by a commercial laboratory (Heartland Assays LLC). Plasma total 25(OH)D [25-hydroxyvitamin D_2 + 25-hydroxyvitamin D_3] concentrations are reported. For an interim safety analysis, plasma total 25(OH)D concentrations were determined in the initial 95 subjects at Emory University with the use of a chemiluminescent-based automated machine (IDS-iSYS; Immunodiagnostic System). Both vitamin D analytic laboratories participate in the Vitamin D External Quality Assessment Scheme and the National Institute of Standards and Technology/NIH Vitamin D Metabolites QA Program (VitDQAP).

Random assignment and blinding

After written informed consent was obtained, patients were randomly assigned to receive conventional antituberculosis treatment plus oral vitamin D_3 therapy or conventional antituberculosis treatment plus a placebo with the use of a one-to-one treatment-allocation ratio. Treatment assignments were generated by the Emory University–based study biostatistician and implemented locally through a distributed study database with the use of a randomized permuted block algorithm stratified by clinical center site. All study medication bottles had a unique bottle number to allow for blinded dispensing.

The study drugs vitamin D_3 and the placebo were identical in shape and color (Biotech Pharmacal Inc.). Study subjects randomly

assigned to receive vitamin D_3 were given 50,000 IU (1.25 mg) vitamin D_3 orally 3 times weekly for 8 consecutive weeks followed by 50,000 IU vitamin D_3 orally every 2 wk for an additional 8 wk. The high-dose vitamin D bolus regimen was modeled after other high-dose vitamin D clinical practice regimens and studies in adults that safely maintained 25(OH)D blood concentrations into a range >75 nmol/L (26). Plasma 25(OH)D concentrations <50 nmol/L were considered to represent vitamin D deficiency (27).

Dietary intake of vitamin D

Dietary vitamin D intake was estimated from a 3-d food intake questionnaire at baseline, week 8, and week 16, and mean daily intake was calculated (28). The validated food-intake instrument was designed to capture the composition of specific foods, including those common in Georgian culture, and was developed specifically for this tuberculosis patient population (28).

*Taq*I vitamin D receptor polymorphisms

Blood DNA was isolated from the final 115 study subjects enrolled with the use of the Mag-Bind SQ Blood DNA Isolation Kit (Omega BioTek). Genomic DNA was quantitated and genotyped for *Taq*I polymorphisms of the vitamin D receptor (VDR) (*tt*, *TT*, and *Tt* genotypes, respectively) with the use of TaqMan chemistry (Applied Biosystems).

Safety monitoring

Criteria for subject discontinuation were established pre hoc. Subjects were withdrawn from the study drug (but followed per protocol in the intent-to-treat-safety analysis) if a plasma calcium concentration was >2.6 mmol/L with signs and symptoms of hypercalcemia at follow-up visits or if a plasma calcium concentration was >2.9 mmol/L at any visit regardless of the presence of symptoms. Study subjects were also queried for clinical symptoms that could potentially be related to hypercalcemia with a specific case report form (CRF) at weeks 2, 4, 8, 12, and 16. An Emory University–based endocrinologist, who was unrelated to the study, served as medical monitor and evaluated all safety-related data after the first 123 subjects were enrolled.

Data collection and management

Research data were entered into a paper CRF and transcribed into a web-based CRF that allowed for a real-time evaluation of data by Emory-based investigators.

Sample size calculation and statistical analyses

A sample size of 110 participants per treatment arm provided 84% power to detect a difference of 15% in the proportion of participants with a culture conversion from positive to negative at 8 wk (2-sided z test with pooled variance; significance level: 0.05). This power calculation was based on the article by Nursyam et al. (17) and assumed that 75% of participants who received conventional antituberculosis treatment plus the placebo were culture negative after 8 wk of treatment and an increase of 15% in culture conversion to 90% in the conventional antituberculosis treatment plus oral vitamin D_3 group.

The primary safety analysis included data from all randomly assigned subjects (n = 199). Efficacy analyses excluded 7 patients with suggestive symptoms of pulmonary tuberculosis disease whose baseline (day 0) prestudy drug sputum culture was later found to be negative for *Mtb*.

The primary efficacy endpoint was the time to sputum culture conversion estimated for each patient as the midpoint between the last positive *Mtb* sputum culture and the first negative sputum culture according to the method of Martineau et al. (19). The secondary clinical outcome variable was the sputum culture conversion at 8 wk. A cumulative culture-conversion percentage was estimated with the use of the Kaplan-Meier method. Log-rank tests were used to compare culture conversion over time by treatment group and by baseline demographic and clinical characteristics. HRs were calculated to measure the degree of association between baseline covariates and culture conversion by fitting the Cox proportional-hazards regression model. Factors that were significant at $P < 0.05$ in the univariable analyses (sex and MDR-TB status) plus the treatment group, age at random assignment, and baseline sputum smear positivity characteristics were included in multivariable analyses (29). The HR and its 95% CI were calculated for each factor in the presence of others in the final model.

A post hoc Cox regression analysis was performed to determine whether the effect of treatment on the time to sputum culture conversion was modified on the basis of MDR-TB status by testing the statistical interaction between the treatment group and MDR-TB status as outlined previously (29). We also determined, as secondary endpoints in this intention-to-treat trial, the effect on culture conversion of a high-dose vitamin D treatment in light of several potential disease modifiers in this cohort. These subgroup analyses included cumulative sputum conversion by treatment group in 59 subjects without MDR-TB who exhibited frank vitamin D deficiency [25(OH)D concentration <25 nmol/L]; in the 169 subjects without MDR-TB with the exclusion of 7 additional subjects who were culture negative at baseline; as a function of stratification by *Taq*I VDR polymorphisms (*tt*, *TT*, and *Tt* genotypes, respectively); in subjects above or below the median value for baseline plasma 25(OH)D concentration; as a function of the season of recruitment (December to February, March to May, June to August, and September to November, respectively), as a function of cavitary disease on baseline chest radiograph (yes or no), or as a function of the level of baseline sputum positivity score (≥4 compared with all other scores). Additional details on statistical methods are provided in the **Supplemental Material**.

RESULTS

Subjects

A total of 784 AFB sputum smear-positive subjects were assessed for eligibility. Of these individuals, 345 subjects were excluded for not meeting the inclusion or exclusion criteria, and 240 additional subjects declined to participate (**Figure 1**). A total of 199 subjects were randomly assigned to receive either high-dose vitamin D_3 (n = 100) or a placebo (n = 99). The study was stopped when the funding provided to perform the major aspects of the study was fully used.

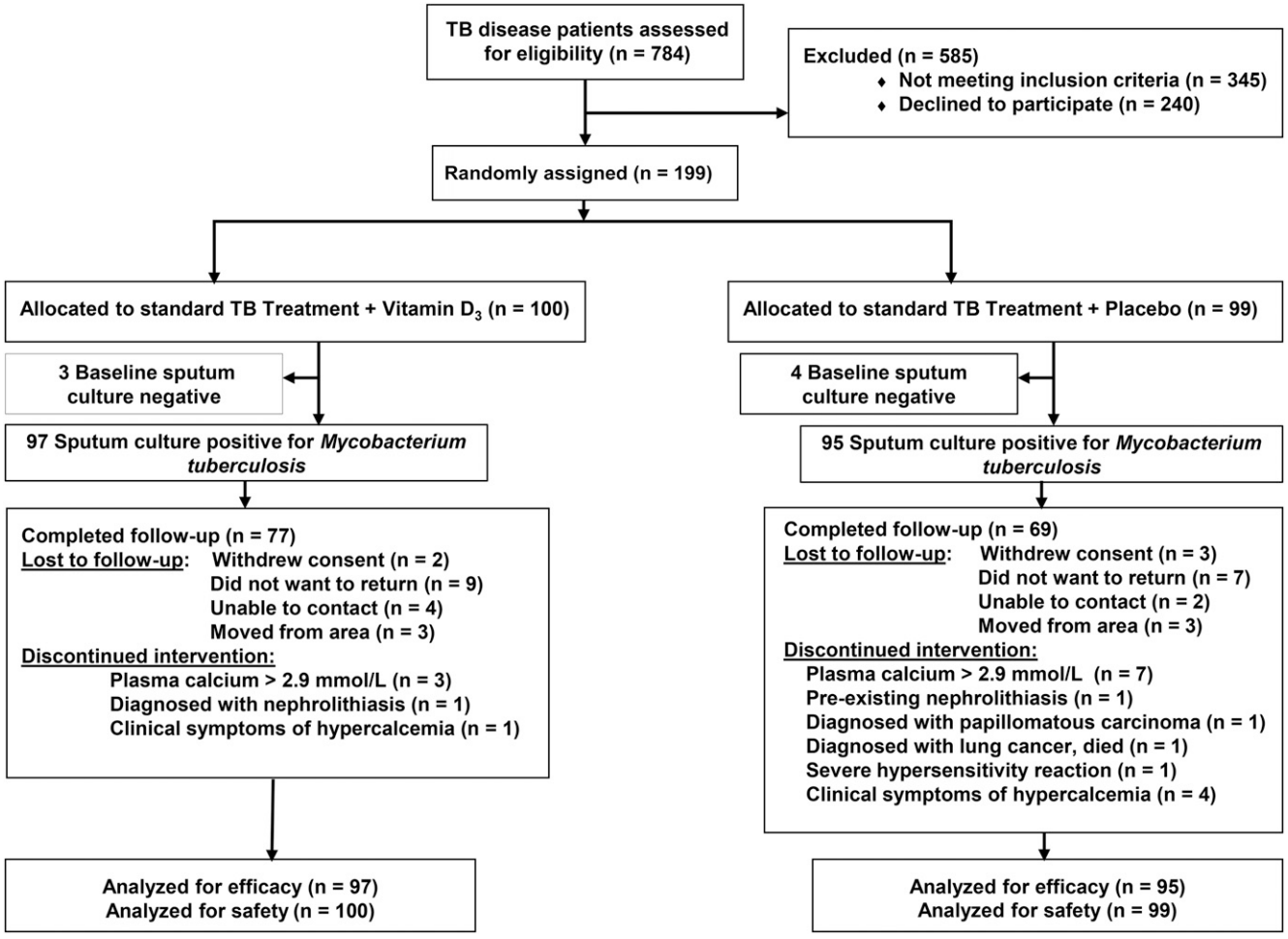

FIGURE 1 Consolidated Standards of Reporting Trials diagram of the progress through the phases (enrollment, intervention allocation, follow-up, and data analysis) of the double-blind randomized controlled trial that compared standard antimicrobial TB treatment plus high-dose vitamin D_3 or standard antimicrobial TB treatment plus a placebo. TB, tuberculosis.

Demographic and clinical characteristics

Demographic and clinical characteristics were comparable between study groups (**Table 1**). Most subjects (92%) were of Georgian ethnicity. A total of 191 of 199 subjects were tested for HIV co-infection; 3 subjects who were randomly assigned to the placebo group (none with MDR-TB) and one subject with MDR-TB who was randomly assigned to the vitamin D_3 group were HIV positive. The *Taq*I genotypes of VDR polymorphism were distributed similarly between the 2 study groups (Table 1).

Vitamin D status and impact of high-dose vitamin D_3 administration

A similar number of subjects in the placebo (71%) and vitamin D_3 (77%) groups were vitamin D deficient (<50 nmol/L or <20 ng/mL) at baseline (NS) (Table 1, **Figure 2**A). Overall, 37% of subjects were severely vitamin D deficient (<25 nmol/L or <10 ng/mL), and 90% of subjects could be classified as vitamin D insufficient (<75 nmol/L or <30 ng/mL); these categories of vitamin D nutriture were similar between treatment groups at baseline (Table 1). As expected, mean 25(OH)D concentrations in placebo-treated subjects remained in the deficient range throughout the 16-wk study. In contrast, high-dose vitamin D_3 resulted in a significant increase in plasma 25(OH)D concentrations to 175, 225, and 250 nmol/L at study weeks 2, 4, and 8, respectively (Figure 2A). During the latter 8 wk of the trial, plasma 25(OH)D concentrations declined to ~175 and 125 nmol/L at weeks 12 and 16, respectively. Reported dietary intake of vitamin D was similar between groups and did not significantly differ over time (data not shown).

Effect of high-dose vitamin D_3 and other variables on sputum *Mtb* culture conversion

There was no significant difference in the median time to culture conversion between subjects who received standard antituberculosis therapy plus high-dose vitamin D_3 (29 d; 95% CI: 24, 36 d) and those who received standard antituberculosis therapy plus the placebo (27 d; 95% CI: 23, 36 d) (P-unadjusted = 0.99; log-rank test) (Figure 2B). There was no significant difference between vitamin D_3 and placebo groups in overall sputum conversion (through week 16) (**Table 2**) and sputum conversion at the 8-wk time point (Supplemental Table 1). Female sex was significantly associated with a more-rapid rate of culture conversion and an overall higher rate of sputum conversion (P = 0.01); as expected, MDR-TB was significantly associated with

TABLE 1
Demographic and clinical characteristics by treatment allocation at baseline[1]

Characteristic	Standard tuberculosis treatment + placebo (n = 99)	Standard tuberculosis treatment + vitamin D_3 (n = 100)
Age,[2] y	34.1 ± 12.4 (18, 63)	32.4 ± 10.6 (18, 62)
Sex, n (%)		
M	60 (60.6)	67 (67.0)
F	39 (39.4)	33 (33.0)
BMI, kg/m^2, n (%)		
<18.5	28 (28.3)	18 (18.0)
≥18.5	71 (71.7)	82 (82.0)
Yearly subject income, Georgian lari (US$586), n (%)		
<1000	27 (27.3)	31 (31.0)
≥1000	72 (72.7)	69 (69.0)
Employment status, n (%)		
Yes	49 (49.5)	42 (42.0)
No	50 (50.5)	58 (58.0)
Current smoker, n (%)		
Yes	34 (34.3)	40 (40.0)
No	65 (65.7)	60 (60.0)
Diabetes mellitus history, n (%)		
Yes	7 (7.1)	3 (3.0)
No	92 (92.9)	97 (97.0)
Chest radiograph, n (%)		
Cavitation absent	78 (78.8)	85 (85.0)
Cavitation present	21 (21.2)	15 (15.0)
Sputum smear, AFB,[3] n (%)		
+1 or +2	79 (79.8)	90 (90.0)
+3 or +4	20 (20.2)	10 (10.0)
Isoniazid and rifampicin resistant,[4] n (%)		
Yes	11 (11.1)	12 (12.0)
No	88 (88.9)	88 (88.0)
Plasma 25(OH)D, ng/mL, n (%)		
<10	35/98 (36)	37/98 (38)
≥10	63/98 (64)	61/98 (62)
<20	71/98 (72)	76/98 (78)
≥20	27/98 (28)	22/98 (22)
<30	90/98 (92)	90/98 (92)
≥30	8/98 (8)	8/98 (8)
*Taq*I genotype,[5] n (%)		
tt	13/59 (22.0)	17/56 (30.4)
TT	21/59 (35.6)	12/56 (21.4)
Tt	25/59 (42.4)	27/56 (48.2)

[1]A total of 4 subjects of 191 subjects tested had HIV co-infection (3 subjects randomly assigned to the placebo group and one subject randomly assigned to the vitamin D_3 group). HIV status was unknown in 8 subjects at entry and during the study. The chi-square or Fisher's exact test was used for comparisons between groups. AFB, acid-fast bacilli; 25(OH)D, 25-hydroxyvitamin D.

[2]All values are means ± SDs; minimums, maximums in parentheses.

[3]Criteria were as follows: +1 denotes 1–9 AFB observed/100 high-powered fields; +2 denotes 1–9 AFB/10 high-powered fields; +3 denotes 1–9 AFB/high-powered field; and +4 denotes >9 AFB/high-powered field.

[4]Multidrug-resistant tuberculosis.

[5]n = 115 (placebo group: n = 59; vitamin D_3 group: n = 56).

a delay in sputum culture conversion (P = 0.002) (Table 2). There was no significant effect on culture conversion as a function of the median age, major dichotomized baseline characteristics, or *Taq*I genotype status (*tt*, *TT*, and *Tt*) (Table 2).

In a Cox regression analysis of baseline risk factors associated with the time to sputum culture conversion, there were no significant differences between vitamin D_3 and placebo groups in univariate or multivariate analyses (**Table 3**). In the multivariate analysis, female sex was associated with an increased rate of culture conversion (adjusted HR: 1.68; 95% CI: 1.19, 2.36; P = 0.003), and the presence of MDR-TB was associated with delayed culture conversion (adjusted HR: 0.37; 95% CI: 0.21, 0.65; $P < 0.001$) (Table 3).

Subgroup analysis

We carried out 2 initial post hoc subgroup analyses to assess the impact of high-dose vitamin D_3 supplementation in individuals with MDR-TB and in those with VDR *Taq*I genotype status. There was heterogeneity of the effects of vitamin D_3 treatment as a function of MDR-TB status. Cox-model results for the effect of treatment allocation showed a strong tendency for this to be modified by MDR-TB status (P = 0.065; test for interaction between treatment and MDR-TB status. Vitamin D_3 administration quantitatively shortened the time to sputum culture conversion in the 12 study subjects with MDR-TB who received high-dose vitamin D_3 compared with that in the 11 subjects who received placebo (HR: 2.01; 95% CI: 0.71, 5.68; P = 0.19), but this result was NS.

There was no effect of baseline VDR *Taq*I genotype status (n = 115) on the time to sputum culture conversion or the effect of vitamin D_3 administration (Tables 2 and 3, Supplemental Table 1). Data on culture conversion at the 8-wk time point was available for 18 MDR-TB patients (8 subjects in the vitamin D_3 group and 10 subjects in the placebo group); again, there was a strong trend toward higher culture conversion at 8 wk in subjects who received vitamin D_3 than for those who received the placebo (87.5% compared with 40%; P = 0.07) (**Supplemental Table 2**). There was no significant difference between groups in the time to starting second line drugs after study entry (placebo: 51.4 d; vitamin D_3: 61.6 d; NS).

Additional post hoc subgroup analysis revealed no significant effect of vitamin D_3 administration on cumulative sputum conversion in the 59 subjects without MDR-TB who exhibited frank vitamin D deficiency [25(OH)D concentration <25 nmol/L] at baseline; in the 169 subjects without MDR-TB with the exclusion 7 additional subjects who were culture-negative at baseline; as a function of stratification by *Taq*I VDR polymorphisms (*tt*, *TT*, and *Tt* genotypes, respectively); as a function of being above or below the median value for the baseline plasma 25(OH)D concentration; as a function of season of recruitment (December to February, March to May, June to August, and September to November, respectively), as a function of cavitary disease on baseline chest radiograph (yes or no), or as a function of the level of baseline sputum positivity score (≥4 compared with all other scores) (data not shown).

Safety of high-dose vitamin D_3

Adverse events were similar between vitamin D_3 and placebo groups. The medical monitor identified no safety concerns after

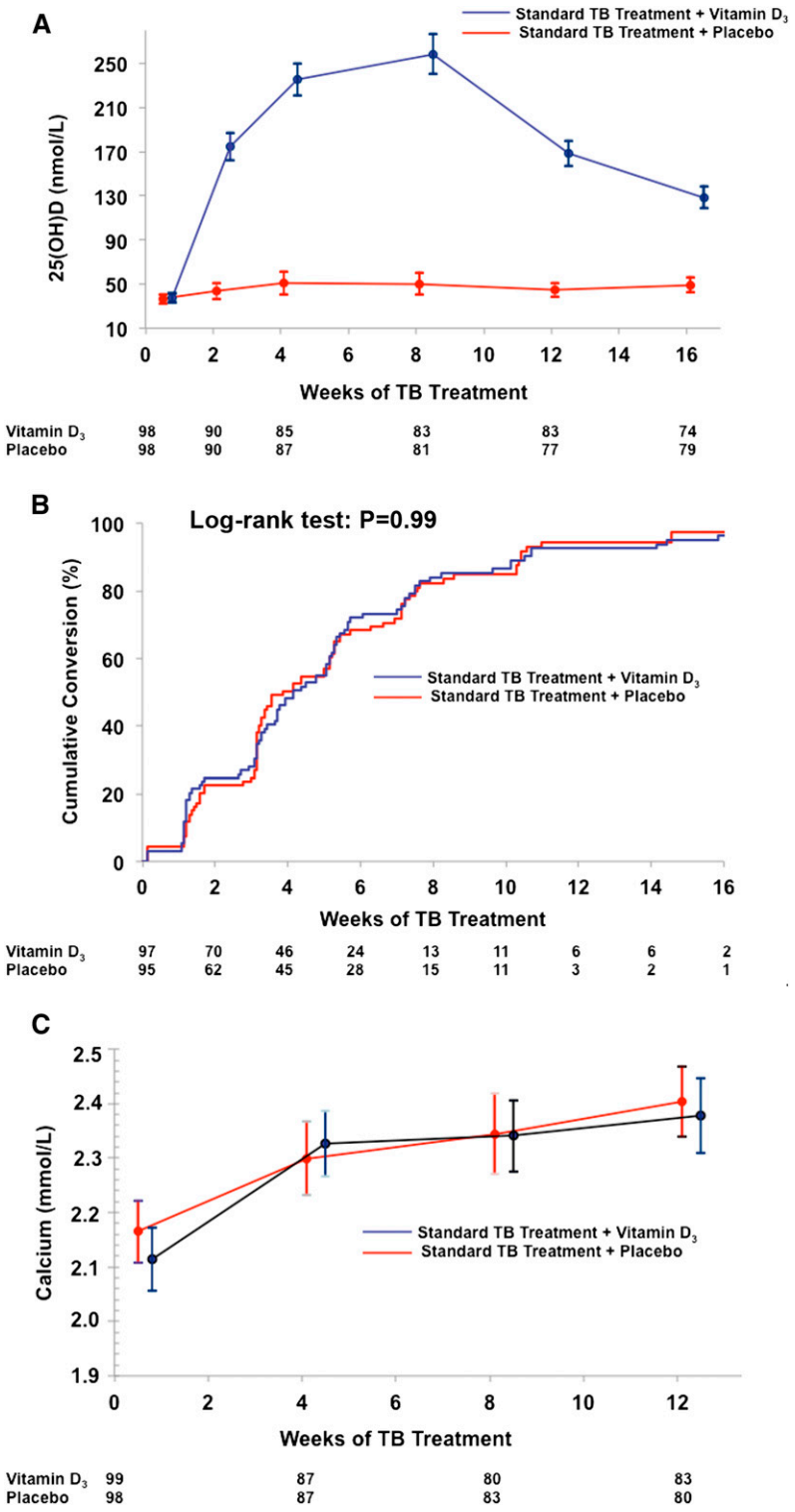

FIGURE 2 Cumulative culture conversion and longitudinal changes in plasma vitamin D and plasma calcium by treatment group. (A) Model-based mean (95% CI) longitudinal changes in mean plasma 25-hydroxyvitamin D concentrations (ng/mL) by treatment group. (B) Cumulative rates of culture conversion in 192 culture-positive pulmonary tuberculosis-disease subjects (n = 97 received standard anti-TB drug therapy plus high-dose vitamin D_3 and n = 95 received standard anti-TB drug therapy plus a placebo). Numbers of patients with a positive culture who remained under follow-up (number of patients at risk) at baseline and study weeks 2, 4, 6, 8, 12, and 16 are shown below the days of antimicrobial treatment. (C) Model-based mean (95% CI) longitudinal changes in mean plasma calcium concentrations (mg/dL) by treatment group at baseline and study weeks 4, 8, and 12. Cumulative culture conversion percentages were estimated with the use of the Kaplan-Meier method. Repeated-measures analyses of plasma calcium and plasma vitamin D_3 were done with a means model (SAS Proc Mixed, version 9.3; SAS Institute Inc.), which provided separate estimates of means by the time of the study (baseline and 2, 4, 8, 12, and 16 wk) and treatment group. Numbers below time points signify the number of subjects in each study group. TB, tuberculosis; 25(OH)D, 25-hydroxyvitamin D.

TABLE 2
Cumulative sputum culture conversion according to baseline characteristics[1]

Characteristic	*n*	Conversion frequency, *n* (%)	Cumulative culture conversion, % (95% CI)		Log-rank *P*
			28 d	56 d	
All subjects	192	169 (88.0)	49.2 (41.9, 56.5)	83.1 (77.5, 88.6)	—
Treatment					0.99
Standard tuberculosis therapy + placebo	95	84 (88.4)	50.2 (39.9, 60.4)	82.1 (74.1, 90.2)	
Standard tuberculosis therapy + vitamin D_3	97	85 (87.6)	48.2 (37.9, 58.5)	84.0 (76.2, 91.7)	
Age, y					0.83
Less than the median of 30	95	81 (85.3)	48.0 (37.6, 58.4)	86.3 (78.9, 93.7)	
At least the median of 30	97	88 (90.7)	50.3 (40.2, 60.5)	80.0 (71.7, 88.2)	
Sex					0.01
M	123	105 (85.4)	43.2 (34.2, 52.3)	79.1 (71.5, 86.7)	
F	69	64 (92.8)	59.4 (47.7, 71.2)	89.7 (82.3, 97.1)	
BMI, kg/m^2					0.92
<18.5	46	40 (87.0)	48.8 (33.8, 63.7)	84.8 (73.7, 95.8)	
≥18.5	146	129 (88.4)	49.4 (41.1, 57.7)	82.6 (76.1, 89.1)	
Yearly subject income, Georgian lari (US$586)					0.69
<1000	55	49 (89.1)	49.6 (36.0, 63.2)	86.4 (77.1, 95.8)	
≥1000	137	120 (87.6)	49.1 (40.5, 57.7)	81.6 (74.8, 88.5)	
Employment status					0.96
Yes	87	78 (89.7)	48.3 (37.5, 59.1)	82.3 (74.0, 90.7)	
No	105	91 (86.7)	50.0 (40.1, 59.8)	83.6 (76.1, 91.1)	
Current smoker					0.46
Yes	73	59 (80.8)	52.1 (40.1, 64.2)	80.2 (70.2, 90.2)	
No	119	110 (92.4)	47.5 (38.4, 56.6)	84.6 (77.9, 91.2)	
Diabetes mellitus history					0.11
Yes	10	10 (100)	70.0 (41.6, 98.4)	90.0 (71.4, 100.0)	
No	182	159 (87.4)	48.0 (40.5, 55.5)	82.7 (76.9, 88.5)	
Chest radiograph					0.35
Cavitation present	36	34 (94.4)	57.1 (40.7, 73.5)	88.6 (78.0, 99.1)	
Cavitation absent	156	135 (86.5)	47.3 (39.3, 55.4)	81.7 (75.3, 88.1)	
Sputum smear, AFB[2]					0.33
+1 or +2	162	144 (88.9)	50.1 (42.2, 58.0)	83.5 (77.5, 89.5)	
+3 or +4	30	25 (83.3)	44.7 (26.6, 62.8)	80.8 (66.0, 95.6)	
Isoniazid and rifampicin resistant[3]					0.002
Yes	23	15 (65.2)	36.6 (16.1, 57.1)	52.5 (30.6, 74.3)	
No	169	154 (91.1)	50.7 (43.0, 58.4)	86.8 (81.5, 92.2)	
Plasma 25-hydroxyvitamin D, ng/mL					0.92
<20	142	124 (87.3)	49.4 (41.0, 57.9)	82.3 (75.7, 89.0)	
≥20	48	44 (91.7)	49.7 (35.2, 64.1)	84.7 (74.2, 95.1)	
*Taq*I genotype[4]					0.44
tt	27	24 (88.9)	44.4 (25.7, 63.2)	82.7 (68.0, 97.4)	
TT	33	33 (100)	60.6 (43.9, 77.3)	87.9 (76.7, 99.0)	
Tt	51	49 (96.1)	49.0 (35.3, 62.7)	80.4 (69.5, 91.3)	

[1]Efficacy (modified intent-to-treat) was analyzed after the exclusion of 7 patients whose baseline sputum culture was negative for *Mycobacterium tuberculosis*. Log-rank tests were used to compare culture conversion over time by treatment group and by baseline demographic and clinical characteristics. HRs were calculated to measure the degree of association between the baseline covariates and culture conversion by fitting the Cox proportional-hazards regression model. HRs (95% CIs) were calculated for each factor in the presence of others in the final model.

[2]AFB, acid-fast bacilli. Criteria were as follows: +1 denotes 1–9 AFB observed/100 high-powered fields; +2 denotes 1–9 AFB/10 high-powered fields; +3 denotes 1–9 AFB/high-powered field; and +4 denotes >9 AFB/high-powered field.

[3]Multidrug-resistant tuberculosis.

[4]n = 115 (placebo: n = 59; vitamin D_3: n = 56).

evaluating safety-related data at the study midpoint. Severe adverse events were similar between groups (**Supplemental Table 3**). Only one subject died by the 6-mo time point after entry; this subject, in the vitamin D_3 group, withdrew after 18 d because of an inability to travel and died on day 56 (cause of death unknown). Another vitamin D_3–treated subject developed type 2 diabetes mellitus during the study. Neither of these severe adverse events were deemed to be attributable to the study drug. Serial plasma calcium concentrations between the 2 study groups were similar at baseline and at weeks 4, 8, and 12 (Figure 2C). Overall, 20 (10.1%) of 199 study subjects had the study drug discontinued for safety concerns (**Table 4**). There was no significant difference in the rate of hypercalcemia (>2.9 mmol/L) between the placebo group (7%) and vitamin D_3 group (3%)

TABLE 3
Cox regression analysis of baseline factors associated with sputum culture conversion[1]

		Univariate analysis		Multivariate analysis	
Characteristic	*n*	HR (95% CI)	*P*	HR (95% CI)	*P*
Treatment					
Standard tuberculosis therapy + vitamin D_3	97	1.00 (0.74, 1.35)[2]	0.99	0.86 (0.63, 1.18)	0.33
Standard tuberculosis therapy + placebo	95	Reference category	—	—	—
Age, y	192	1.00 (0.98, 1.01)	0.48	1.00 (0.98, 1.01)	0.74
Sex					
F	69	1.48 (1.08, 2.03)	0.0140	1.68 (1.19, 2.36)	0.003
M	123	Reference category	—	—	—
BMI, kg/m^2					
<18.5	46	0.98 (0.69, 1.40)	0.92	—	—
≥18.5	146	Reference category	—	—	—
Yearly subject income, Georgian lari (US$586)					
<1000	55	1.07 (0.77, 1.49)	0.70	—	—
≥1000	137	Reference category	—	—	—
Employment status					
Yes	87	1.01 (0.74, 1.37)	0.96	—	—
No	105	Reference category	—	—	—
Current smoker					
Yes	73	0.89 (0.65, 1.22)	0.47	—	—
No	119	Reference category	—	—	—
Diabetes mellitus history					
Yes	10	1.67 (0.88, 3.17)	0.12	—	—
No	182	Reference category	—	—	—
Chest radiograph					
Cavitation present	36	1.20 (0.82, 1.74)	0.36	—	—
Cavitation absent	156	Reference category	—	—	—
Sputum smear, AFB[2]					
+1 or +2	162	1.20 (0.80, 1.88)	0.34	1.14 (0.74, 1.75)	0.56
+3 or +4	30	Reference category	—	—	—
Isoniazid and rifampicin resistant[3]					
Yes	23	0.45 (0.26, 0.77)	0.0036	0.37 (0.21, 0.65)	0.0006
No	169	Reference category	—	—	—
Plasma 25-hydroxyvitamin D, ng/mL					
<20	142	1.02 (0.72, 1.44)	0.92	—	—
≥20	48	Reference category	—	—	—
*Taq*I genotype[4]					
Tt	51	1.04 (0.64, 1.70)	0.88	—	—
TT	33	1.34 (0.78, 2.28)	0.29	—	—
tt	27	Reference category	—	—	—

[1]Efficacy (modified intent-to-treat) was analyzed after the exclusion of 7 patients whose baseline sputum culture was negative for *Mycobacterium tuberculosis*. Log-rank tests were used to compare culture conversion over time by treatment group and by baseline demographic and clinical characteristics. HRs were calculated to measure the degree of association between baseline covariates and culture conversion by fitting the Cox proportional-hazards regression model. HRs (95% CIs) were calculated for each factor in the presence of others in the final model. Factors that were significant at $P < 0.05$ in the univariable analyses (sex and multidrug-resistant tuberculosis status) plus treatment group, age at random assignment, and baseline sputum smear-positivity characteristics were included in multivariable analyses (29).

[2]AFB, acid-fast bacilli. Criteria were as follows: +1 denotes 1–9 AFB observed/100 high-powered fields; +2 denotes 1–9 AFB/10 high-powered fields; +3 denotes 1–9 AFB/high-powered field; and +4 denotes >9 AFB/high-powered field.

[3]Multidrug-resistant tuberculosis.

[4]$n = 111$ (placebo: $n = 58$; vitamin D_3: $n = 53$).

($P = 0.21$) (Table 4). There were no differences between groups for the incidence of any specific adverse event symptom (**Supplemental Table 4**)

DISCUSSION

This study confirmed a high rate of vitamin D deficiency in Georgian patients with tuberculosis as has been documented in other countries (11–13). Adjunctive high-dose oral vitamin D_3 was safe and led to a substantial increase in plasma 25(OH)D concentrations over 16 wk. However, this effect was not associated with enhanced sputum *Mtb* clearance. These data imply that a high-dose regimen of vitamin D_3 that effectively increases and sustains 25(OH)D concentrations does not improve the efficacy of antituberculosis drugs in patients with newly diagnosed tuberculosis disease.

Our cohort was representative of patients with tuberculosis disease treated at the Tbilisi NCTLD (3, 4, 25). Georgia is

TABLE 4
Study drug discontinuation because of safety concerns

Characteristic	Standard tuberculosis treatment + placebo (n = 99), n (%)	Standard tuberculosis treatment + vitamin D_3 (n = 100), n (%)	P
Hypercalcemia (plasma calcium concentration >2.9 mmol/L)	7 (7)	3 (3)	0.21[1]
Pre-existing nephrolithiasis	1 (1)	0 (0)	—
Diagnosed with prevalent nephrolithiasis	0 (0)	1 (1)	—
Diagnosed with papillomatous carcinoma	1 (1)	0 (0)	—
Diagnosed with pulmonary carcinoma and died	1 (1)	0 (0)	—
Severe hypersensitivity reaction	1 (1)	0 (0)	—
Clinical symptoms of hypercalcemia	4 (4)	1 (1)	—
Any safety event	15 (15)	5 (5)	0.017

[1]Fisher's exact test.

a country with a high burden of MDR-TB but a low rate of HIV infection (1, 3); thus, potential confounding effects of tuberculosis and HIV co-infection on the efficacy of high-dose vitamin D_3 were not factors that influenced our results.

Strengths of the study included the double-blind, intention-to-treat design, an effective vitamin D_3 dosing regimen, and the use of serial sputum *Mtb* cultures as the primary endpoint (1, 3, 4, 24). To our knowledge, our vitamin D_3 dosing regimen is the highest dose used to date in a tuberculosis patient RCT. We confirmed low dietary vitamin D intake coupled with low serum 25(OH)D concentrations, which suggested the need for routine vitamin D monitoring and supplementation in Georgian adults with tuberculosis (14, 28).

The lack of an effect of high-dose vitamin D_3 to accelerate sputum *Mtb* clearance is consistent with the overall findings of a recent double-blind RCT in 146 tuberculosis-disease patients in London (19) and a recent double-blind RCT in 247 tuberculosis patients in India (23). These latter 2 RCTs used a vitamin D_3 dosing regimen of 2.5 mg orally at baseline, which was repeated at weeks 2, 4, and 6, for a total dose of 10 mg (400,000 IU). Thus, our dosing regimen was 3.5-fold higher than in these studies (35 mg or 1.4M IU over 16 wk), and the 25(OH)D concentrations achieved were considerably higher than the concentrations observed in those trials (19, 23) Overall, published data on the impact of adjunctive vitamin D administration in patients with pulmonary tuberculosis are difficult to interpret because of uncertain random assignment (17), varying vitamin D doses and dosing schedules (18–22), the absence of measured blood 25(OH)D concentrations (17, 21), the inability of the vitamin D dose used to increase serial blood 25(OH)D concentrations (18), a lack of data on the impact of vitamin D on sputum culture conversion (17, 18, 20, 22), and/or missing data (23). Our study differed from most previous reports in that we incorporated serial sputum culture results, semiquantitative sputum smear data, baseline vitamin D status, and the presence of cavitation on chest radiographs in our analysis, and we used a higher dose of vitamin D_3 (Figure 2A). The observed difference of 5.5% in the culture-conversion rate at 8 wk (90.4% in the vitamin D_3 arm and 84.9% in the placebo arm) was not felt to be a clinically important difference. To design a trial to detect a difference of 5% in culture conversion at 8 wk would require >500 patients per treatment group to ensure 80% power.

Several cross-sectional studies in different regions of the world have shown variable associations with the presence of specific VDR polymorphisms and susceptibility to tuberculosis disease (30). In contrast with a previous report (19), the *tt* VDR genotype or the presence of the *t* allele (not shown) did not appear to influence sputum conversion at week 8 or over time with or without high-dose vitamin D. Our trial was not specifically powered for genetic studies on VDR polymorphisms; blood was collected for this analysis after being informed by the article of Martineau et al. (19), which was published after the initiation of this RCT. We did not explore the influence of other VDR polymorphisms.

In a post hoc subgroup analysis, we showed an interesting and unexpected strong trend (P = 0.065) for an interaction of MDR-TB status to favorably modify the efficacy of high-dose vitamin D_3 on sputum culture conversion over time in 23 MDR-TB patients. In the 18 subjects with available baseline and week 8 sputum culture data, there was also a nonsignificant trend toward enhanced culture conversion at this time point (P = 0.07) (Supplemental Table 2). The initiation of second-line drug therapy for MDR-TB did not appear to influence the response to vitamin D_3 because this was begun at similar times after the start of therapy (placebo: 51 d; vitamin D_3: 62 d; NS).

Enhanced vitamin D–mediated innate immunity was shown in *Mtb*-infected human monocytes via the transcriptional activation of the antimicrobial peptide LL-37 (5). It is possible that such endogenous immune-stimulating effects may have contributed to sputum-clearance responses in subjects with MDR-TB. Nonetheless, because of the small cohort of MDR-TB subjects, the results are only hypothesis generating. Future RCTs that build on previous studies of systemic innate and adaptive immunity and ex vivo cellular immune responses to high-dose vitamin D_3 (10, 22) in subjects with MDR-TB are of interest to determine whether immune functions are favorably affected by vitamin D_3.

Female sex was associated with significantly enhanced culture conversion over time independent of adjunctive vitamin D_3 intervention (Table 3). There has been little information published on sex-specific effects of the rates sputum culture conversion in tuberculosis-disease patients (31). Thus, enhanced sputum conversion rates in female patients with tuberculosis in Georgia should be further explored in larger cohorts.

Because of our high vitamin D_3 dose, we used strict inclusion and exclusion criteria, serial calcium amounts (not corrected for plasma albumin concentrations), standardized clinical symptom monitoring, the evaluation of safety data by an endocrinologist medical monitor, and pre hoc–determined dropout criteria to minimize risk. We showed that the incidences of hypercalcemia and study-drug discontinuation because of safety concerns were higher in the placebo group than in the vitamin D_3 group. Urinary calcium corrected for creatinine, which is a sensitive marker of potential vitamin D toxicity, was not determined; nonetheless,

the multiple safety indexes we used showed no signal for adverse effects that were attributable to this high-dose vitamin D_3 regimen.

There were several limitations in this study. The design was a short-term 16-wk trial in which tuberculosis-treatment outcomes and a subgroup analysis within the small number of MDR-TB subjects (and the other subgroup analyses previously outlined) were not pre hoc–planned endpoints, which potentially introduced risk of type 1 error. Also, the study was initiated before the institution of rapid molecular testing for MDR-TB in Georgia; thus, we have no data on the effect of vitamin D_3 in MDR-TB patients who were initiated with second-line drugs early in their treatments. We used solid *Mtb*-culture media in all subjects, which has a lower sensitivity for tuberculosis disease than does a liquid culture. We experienced a 24% dropout rate (40 patients did not attend the week 16 visit, and 7 patients were culture negative at baseline), which may have introduced bias in the estimation of the culture-conversion rate. Finally, our data cannot be generalized to tuberculosis-disease subjects with HIV co-infection because of the low prevalence of HIV in these Georgian subjects.

In conclusion, this study, in a cohort of newly diagnosed patients in Georgia who primarily had drug-susceptible pulmonary tuberculosis disease, shows that this adjunctive high-dose vitamin D_3 regimen was safe but did not decrease the time to sputum culture *Mtb* conversion. Additional studies are needed to determine whether adjunctive high-dose vitamin D_3 is beneficial in patients with MDR-TB.

We thank Elizabeth Burgess of the Department of Medicine, Division of Endocrinology, Metabolism and Lipids, Emory University School of Medicine, for service as the medical monitor.

The authors' responsibilities were as follows—NT, KAE, JKF, VM, NL, CDR, VT, HMB, and TRZ: designed the research (project conception, development of the overall research plan, and study oversight); NT, ES, MK, GH, RRK, JKF, VM, JAA, LV, LH, VT, HMB, and TRZ: conducted the research (hands-on conduct of the experiments and data collection); KAE and NS: analyzed the data and performed the statistical analysis; NT, KAE, NS, RRK, JKF, JAA, CDR, VT, HMB, and TRZ: wrote the manuscript; and TRZ: had primary responsibility for the final content of the manuscript. None of the authors reported a conflict of interest related to the study.

REFERENCES

1. World Health Organization. WHO global report. Geneva (Switzerland): WHO Global TB Programme; 2014.
2. Dheda K, Gumbo T, Gandhi NR, Murray M, Theron G, Udwadia Z, Migliori GB, Warren R. Global control of tuberculosis: from extensively drug-resistant to untreatable tuberculosis. Lancet Respir Med 2014;2:321–38.
3. Lomtadze N, Aspindzelashvili R, Janjgava M, Mirtskhulava V, Wright A, Blumberg HM, Salakaia A. Prevalence and risk factors for multidrug-resistant tuberculosis in the Republic of Georgia: a population-based study. Int J Tuberc Lung Dis 2009;13:68–73.
4. Kempker RR, Rabin AS, Nikolaishvili K, Kalandadze I, Gogishvili S, Blumberg HM, Vashakidze S. Additional drug resistance in *Mycobacterium tuberculosis* isolates from resected cavities among patients with multidrug-resistant or extensively drug-resistant pulmonary tuberculosis. Clin Infect Dis 2012;54:e51–4.
5. Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, Ochoa MT, Schauber J, Wu K, Meinken C, et al. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science 2006;311:1770–3.
6. Holick MF. Vitamin D deficiency. N Engl J Med 2007;357:266–81.
7. Martineau AR, Wilkinson RJ, Wilkinson KA, Newton SM, Kampmann B, Hall BM, Packe GE, Davidson RN, Eldridge SM, Maunsell ZJ, et al. A single dose of vitamin D enhances immunity to mycobacteria. Am J Respir Crit Care Med 2007;176:208–13.
8. Miller J, Gallo RL. Vitamin D and innate immunity. Dermatol Ther 2010;23:13–22.
9. Fabri M, Stenger S, Shin DM, Yuk JM, Liu PT, Realegeno S, Lee HM, Krutzik SR, Schenk M, Sieling PA, et al. Vitamin D is required for IFN-gamma-mediated antimicrobial activity of human macrophages. Sci Transl Med 2011;3:104ra102.
10. Coussens AK, Wilkinson RJ, Hanifa Y, Nikolayevskyy V, Elkington PT, Islam K, Timms PM, Venton TR, Bothamley GH, Packe GE, et al. Vitamin D accelerates resolution of inflammatory responses during tuberculosis treatment. Proc Natl Acad Sci USA 2012;109:15449–54.
11. Wejse C, Olesen R, Rabna P, Kaestel P, Gustafson P, Aaby P, Andersen PL, Glerup H, Sodemann M. Serum 25-hydroxyvitamin D in a West African population of tuberculosis patients and unmatched healthy controls. Am J Clin Nutr 2007;86:1376–83.
12. Yamshchikov AV, Kurbatova EV, Kumari M, Blumberg HM, Ziegler TR, Ray SM, Tangpricha V. Vitamin D status and antimicrobial peptide cathelicidin (LL-37) concentrations in patients with active pulmonary tuberculosis. Am J Clin Nutr 2010;92:603–11.
13. Martineau AR, Nhamoyebonde S, Oni T, Rangaka MX, Marais S, Bangani N, Tsekela R, Bashe L, de Azevedo V, Caldwell J, et al. Reciprocal seasonal variation in vitamin D status and tuberculosis notifications in Cape Town, South Africa. Proc Natl Acad Sci USA 2011;108:19013–7.
14. Desai NS, Tukvadze N, Frediani JK, Kipiani M, Sanikidze E, Nichols MM, Hebbar G, Kempker RR, Mirtskhulava V, Kalandadze I, et al. Effects of sunlight and diet on vitamin D status of pulmonary tuberculosis patients in Tbilisi, Georgia. Nutrition 2012;28:362–6.
15. Kim JH, Park JS, Cho YJ, Yoon HI, Song JH, Lee CT, Lee JH. Low serum 25-hydroxyvitamin D level: an independent risk factor for tuberculosis? Clin Nutr 2014;33:1081–6.
16. Mehta S, Mugusi FM, Bosch RJ, Aboud S, Urassa W, Villamor E, Fawzi WW. Vitamin D status and TB treatment outcomes in adult patients in Tanzania: a cohort study. BMJ Open 2013;3:e003703.
17. Nursyam EW, Amin Z, Rumende CM. The effect of vitamin D as supplementary treatment in patients with moderately advanced pulmonary tuberculous lesion. Acta Med Indones 2006;38:3–5.
18. Wejse C, Gomes VF, Rabna P, Gustafson P, Aaby P, Lisse IM, Andersen PL, Glerup H, Sodemann M. Vitamin D as supplementary treatment for tuberculosis: a double-blind, randomized, placebo-controlled trial. Am J Respir Crit Care Med 2009;179:843–50.
19. Martineau AR, Timms PM, Bothamley GH, Hanifa Y, Islam K, Claxton AP, Packe GE, Moore-Gillon JC, Darmalingam M, Davidson RN, et al. High-dose vitamin D(3) during intensive-phase antimicrobial treatment of pulmonary tuberculosis: a double-blind randomised controlled trial. Lancet 2011;377:242–50.
20. Kota SK, Jammula S, Kota SK, Tripathy PR, Panda S, Modi KD. Effect of vitamin D supplementation in type 2 diabetes patients with pulmonary tuberculosis. Diabetes Metab Syndr 2011;5:85–9.
21. Ralph AP, Waramori G, Pontororing GJ, Kenangalem E, Wiguna A, Tjitra E, Sandjaja, Lolong DB, Yeo TW, Chatfield MD, et al. L-arginine and vitamin D adjunctive therapies in pulmonary tuberculosis: a randomised, double-blind, placebo-controlled trial. PLoS One 2013;8:e70032.
22. Salahuddin N, Ali F, Hasan Z, Rao N, Aqeel M, Mahmood F. Vitamin D accelerates clinical recovery from tuberculosis: results of the SUCCINCT Study [Supplementary Cholecalciferol in recovery from tuberculosis]. A randomized, placebo-controlled, clinical trial of vitamin D supplementation in patients with pulmonary tuberculosis. BMC Infect Dis 2013;13:22.
23. Daley P, Jagannathan V, John KR, Sarojini J, Latha A, Vieth R, Suzana S, Jeyaseelan L, Christopher DJ, Smieja M, et al. Adjunctive vitamin D for treatment of active tuberculosis in India: a randomised, double-blind, placebo-controlled trial. Lancet Infect Dis 2015;15:528–34.
24. Tukvadze N, Kempker RR, Kalandadze I, Kurbatova E, Leonard MK, Apsindzelashvili R, Bablishvili N, Kipiani M, Blumberg HM. Use of a molecular diagnostic test in AFB smear positive tuberculosis suspects greatly reduces time to detection of multidrug resistant tuberculosis. PLoS One 2012;7:e31563.
25. Tukvadze N, Bablishvili N, Apsindzelashvili R, Blumberg HM, Kempker RR. Performance of the MTBDRsl assay in Georgia. Int J Tuberc Lung Dis 2014;18:233–9.
26. Pepper KJ, Judd SE, Nanes MS, Tangpricha V. Evaluation of vitamin D repletion regimens to correct vitamin D status in adults. Endocr Pract 2009;15:95–103.

27. Ross AC, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK, Durazo-Arvizu RA, Gallagher JC, Gallo RL, Jones G, et al. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. J Clin Endocrinol Metab 2011;96:53–8.
28. Frediani JK, Tukvadze N, Sanikidze E, Kipiani M, Hebbar G, Easley KA, Shenvi N, Ramakrishnan U, Tangpricha V, Blumberg HM, et al. A culture-specific nutrient intake assessment instrument in patients with pulmonary tuberculosis. Clin Nutr 2013;32:1023–8.
29. Wang R, Lagakos SW, Ware JH, Hunter DJ, Drazen JM. Statistics in medicine–reporting of subgroup analyses in clinical trials. N Engl J Med 2007;357:2189–94.
30. Azad AK, Sadee W, Schlesinger LS. Innate immune gene polymorphisms in tuberculosis. Infect Immun 2012;80:3343–59.
31. Karim F, Ahmed F, Begum I, Johansson E, Diwan VK. Female-male differences at various clinical steps of tuberculosis management in rural Bangladesh. Int J Tuberc Lung Dis 2008;12:1336–9.