Clinical Cancer Research

Plasma 25-Hydroxyvitamin D Levels and Survival in Patients with Advanced or Metastatic Colorectal Cancer: Findings from CALGB/SWOG 80405 (Alliance)

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Abstract

Purpose: Previous studies have suggested that higher circulating 25-hydroxyvitamin D [25(OH)D] levels are associated with decreased colorectal cancer risk and improved survival. However, the influence of vitamin D status on disease progression and patient survival remains largely unknown for patients with advanced or metastatic colorectal cancer.

Experimental Design: We prospectively collected blood samples in 1,041 patients with previously untreated advanced or metastatic colorectal cancer participating in a randomized phase III clinical trial of first-line chemotherapy plus biologic therapy. We examined the association of baseline plasma 25(OH)D levels with overall survival (OS) and progression-free survival (PFS). Cox proportional hazards models were used to calculate hazard ratios (HRs) and confidence intervals (CIs), adjusted for prognostic factors and confounders.

Results: At study entry, 63% of patients were vitamin D deficient (<20 ng/mL) and 31% were vitamin D insufficient (20–<30 ng/mL). Higher 25(OH)D levels were associated with an improvement in OS and PFS ($P_{trend} = 0.0009$ and 0.03, respectively). Compared with patients in the bottom quintile of 25(OH)D (≤10.8 ng/mL), those in the top quintile (≥24.1 ng/mL) had a multivariable-adjusted HR of 0.66 (95% CI, 0.53–0.83) for OS and 0.81 (95% CI, 0.66–1.00) for PFS. The improved survival associated with higher 25(OH)D levels was consistent across patient subgroups of prognostic patient and tumor characteristics.

Conclusions: In this large cohort of patients with advanced or metastatic colorectal cancer, higher plasma 25(OH)D levels were associated with improved OS and PFS. Clinical trials assessing the benefit of vitamin D supplementation in patients with colorectal cancer are warranted.

Introduction

Vitamin D deficiency is increasingly prevalent in the United States. A national survey showed that only 23% of Americans have serum 25-hydroxyvitamin D [25(OH)D] levels ≥30 ng/mL, the level required for optimum health (1). Most foods, unless they are fortified, are poor sources of vitamin D. Thus, exposure to type B ultraviolet (UV-B) radiation is the major determinant of vitamin D status in humans. Over the past decades, skin cancer prevention campaigns that recommend avoidance of sun exposure, coupled with more daylight hours spent indoors and increasing prevalence of obesity, may have contributed to the rising prevalence of vitamin D deficiency, particularly in northern latitudes (2).

Colorectal cancer is the second leading cause of cancer-related death in the United States (3). Among patients with colorectal cancer, only 39% are diagnosed at an early stage with a 5-year survival rate of 90%; the survival rates decline to 71% and 14% for locally advanced and metastatic stages, respectively (4). Vitamin D has antineoplastic properties (5), and patients with colorectal cancer are prone to vitamin D deficiency (6–9). Prospective epidemiologic studies consistently show an association between higher vitamin D status and improved survival among patients with all stages of colorectal cancer (8, 10–13). However, the influence of vitamin D status on cancer progression and survival remains largely unknown for patients with advanced or metastatic

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Translational Relevance

Preclinical and epidemiologic evidence indicates that vitamin D has a beneficial effect on colorectal cancer survival. Among 1,041 patients with advanced or metastatic colorectal cancer participating in a randomized clinical trial, we observed a particularly high prevalence of vitamin D deficiency at baseline. Higher plasma 25-hydroxyvitamin D [25(OH)D] levels were associated with a significant improvement in overall and progression-free survival, indicating that vitamin D supplementation to raise 25(OH)D levels may play a role in treatment of advanced and metastatic colorectal cancer. Clinical trials assessing the benefit of vitamin D supplementation in patients with colorectal cancer are warranted.

colorectal cancer. We therefore examined the prevalence of vitamin D deficiency and the association between baseline plasma 25 (OH)D levels and survival outcome in a large cohort of patients with previously untreated advanced or metastatic colorectal cancer enrolled in a randomized phase III clinical trial of first-line chemotherapy plus biologic therapy.

Materials and Methods

Study population

Patients in this study were drawn from the Cancer and Leukemia Group B (CALGB; now a part of the Alliance for Clinical Trials in Oncology) and SWOG 80405 (Alliance) trial, which was designed in collaboration with the NCI to compare various combinations of chemotherapy [leucovorin, fluorouracil, and oxaliplatin (mFOLFOX6) or leucovorin, fluorouracil, and irinotecan (FOLFIRI) per investigator's choice] with biologic therapy as first-line treatment of advanced and metastatic colorectal cancer: (i) chemotherapy plus cetuximab; (ii) chemotherapy plus bevacizumab; and (iii) chemotherapy plus cetuximab and bevacizumab. Patients were enrolled at centers across the National Clinical Trials Network (NCTN) in the United States and Canada. Eligible patients had pathologically confirmed, unresectable, locally advanced or metastatic colorectal cancer. Patients had to be candidates for either mFOLFOX6 or FOLFIRI regimens without known contraindications for bevacizumab or cetuximab therapy. Patients were required to have had no previous treatment for advanced or metastatic disease but may have received prior adjuvant treatment ($\leq$6 months) that must have concluded >12 months before recurrence. Institutional review board approval was required at all participating centers and all participating patients provided written informed consent. The study was conducted in accordance with the International Ethical Guidelines for Biomedical Research Involving Human Subjects (CIOMS).

Full details and results of the treatment trial have been described previously (14). In brief, the trial was initiated in September 2005 with a total of 2,326 patients randomized to the three treatment arms. The lack of efficacy of EGFR antibodies in *KRAS*-mutant tumors (15) and failure of the chemotherapy and dual antibody combination in other studies (16, 17) resulted in a pivotal amendment restricting eligibility to patients with confirmed *KRAS* wild-type tumors in November 2008 and closure of the dual antibody arm in September 2009. Although the final analysis cohort for the treatment trial was composed of only the 1,131 *KRAS* wild-type patients randomized to the bevacizumab-chemotherapy arm or the cetuximab-chemotherapy arm, the study population for this vitamin D study was drawn from all three arms of CALGB/SWOG 80405 (Alliance). Among the 2,326 patients, 1,041 provided blood samples at study entry for biomarker research and had 25(OH)D levels measured (Supplementary Fig. S1). We compared baseline characteristics of these 1,041 patients with the entire population as well as the final analysis cohort for the treatment trial, and did not detect any appreciable differences (Supplementary Table S1). Moreover, patients in both populations experienced similar overall survival (OS) (median = 28.8 and 31.2 months, respectively).

Plasma 25(OH)D assessment

SWOG oversaw specimen biobanking and distribution of samples for correlative research. To measure 25(OH)D, plasma samples were sent by overnight delivery to Heartland Assays for radioimmunoassay (18). Masked quality control samples were interspersed among the samples, and all laboratory personnel were blinded to survival data. The mean intraassay coefficient of variation was 10%, and National Institute of Standards and Technology reference ranges ($\pm$SDs) were met as follows: 23.3 $\pm$ 1.8 ng/mL for concentration 1, 14.6 $\pm$ 1.3 ng/mL for concentration 2, 38.6 $\pm$ 2.4 ng/mL for concentration 3, and 33.1 $\pm$ 2.6 ng/mL for concentration 4.

Clinical outcomes

The primary endpoint of OS was calculated from time of study entry to death or last known follow-up for those without reported death. The secondary endpoint of progression-free survival (PFS) was calculated from study entry to first documented progression or death. Patients alive without documented progression were censored for PFS at the most recent disease assessment. Disease assessment was done by treating investigators and was not blinded.

Covariates

Body mass index (BMI) was calculated from weight and height measured at study entry (weight in kilograms divided by square of height in meters). At enrollment, patients were given the option of inclusion in the diet and lifestyle companion study. Within the first month of enrollment, 774 of the 1,041 patients completed a questionnaire capturing diet and lifestyle habits at diagnosis of advanced or metastatic disease, including a validated semiquantitative food frequency questionnaire that consisted of 131 food items plus vitamin and mineral supplement use. A physical activity score, expressed in metabolic equivalent-hours/week, was derived by multiplying the time spent on each activity per week by the typical energy expenditure for that activity and then summing contributions from all activities. Dietary vitamin D and calcium intakes were computed by multiplying the frequency of consumption of each food by its nutrient content and summing contributions from all foods.

Patients who consented to be tested for *KRAS* agreed to submit two archival paraffin-embedded tumor tissue sections and one histology reference slide or one paraffin-embedded tumor block of previously resected primary colorectal tumor and/or a metastatic tumor deposit. *KRAS* and *NRAS* mutation status was determined by BEAMing (beads, emulsion, amplification, magnetics) technology.

Statistical analyses

Survival curves were generated using the Kaplan–Meier method (19), and statistical significance was measured using the log-rank test (20). Cox proportional hazards models (21) were used to examine the association of 25(OH)D levels with OS and PFS. The assumption of proportional hazards was tested and met by evaluating a time-dependent variable, which was the product of 25(OH)D level and time. Hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated according to (i) quintile of 25(OH)D, with the lowest quintile as the reference group; and (ii) clinical category of 25(OH)D (<10, 10 to <20, or ≥20 ng/mL), with <10 ng/mL as the reference group. We tested for a linear trend using the median value of each quintile as a continuous variable. In multivariable models, we included *a priori* the covariates that are known to be prognostic factors for colorectal cancer survival or related to 25(OH)D levels, including age, sex, race, Eastern Cooperative Oncology Group (ECOG) performance status, *RAS* mutation status, prior adjuvant chemotherapy, chemotherapy backbone, assigned treatment arm, BMI, physical activity, geographic region of residence (as a surrogate for UV-B exposure), and season of blood collection.

We next examined whether the association of 25(OH)D levels with OS and PFS varied according to other prognostic factors. Interactions between 25(OH)D and potential effect modifiers were assessed by entering in the model the cross product of the 25(OH)D level as a continuous variable and the stratification variable, evaluated by the likelihood ratio test.

Data collection was conducted by the Alliance Statistics and Data Center. Data quality was ensured by review of data by the Alliance Statistics and Data Center following Alliance policies. Statistical analysis was performed based on the study database frozen on January 18, 2018, using SAS software, version 9.4 (SAS Institute). All *P* values are two-sided and were considered significant at the 0.05 level.

Results

Among 1,041 patients with advanced or metastatic colorectal cancer, the mean age was 59 years (standard deviation, 12 years), with 58% males and 42% females. The vast majority of patients were white (86%). At study entry, the median plasma 25(OH)D level in the entire study population was 17.2 ng/mL (range, 2.2–72.7 ng/mL) and the mean was 17.7 ng/mL (SD = 7.6 ng/mL); 63% of patients were vitamin D deficient (<20 ng/mL), 31% were vitamin D insufficient (20–<30 ng/mL), and only 6% were vitamin D sufficient (≥30 ng/mL). We also detected a 17% prevalence of extremely low 25(OH)D levels (<10 ng/mL).

Baseline characteristics by quintile of 25(OH)D are shown in Table 1. Patients with higher 25(OH)D levels had a lower BMI, were more likely to be of white race, were more likely to possess an ECOG performance status of 0, were more likely to have *RAS* wild-type tumors (defined as wild-type in both *KRAS* and *NRAS*), and consumed higher levels of total vitamin D and calcium.

The median follow-up time among living patients was 5.6 years (90th percentile: 7.7 years). A total of 987 patients (95%) had died or progressed. Survival curves by quintile of 25(OH)D are shown in Fig. 1 (log-rank *P* comparing extreme quintiles = 0.0004 for OS and 0.02 for PFS). Higher 25(OH)D levels were associated with a significant improvement in OS (P_{trend} = 0.001; Table 2). These results did not change after adjustment for potential confounding factors (P_{trend} = 0.0009). Compared with patients in the bottom quintile of 25(OH)D (≤10.8 ng/mL), those in the top quintile (≥24.1 ng/mL) had a multivariable-adjusted HR for OS of 0.66 (95% CI, 0.53–0.83), corresponding to an 8-month longer median OS time. Similarly, higher plasma 25(OH)D levels were associated with a significant improvement in PFS, with patients in the top quintile having a multivariable-adjusted HR for PFS of 0.81 (95% CI, 0.66–1.00; P_{trend} = 0.03), compared with those in the bottom quintile. In sensitivity analyses, we additionally adjusted for number of metastatic sites and liver metastasis, and the association remained similar. In analyses examining survival by clinical category of 25(OH)D, a similar positive association was noted between 25(OH)D levels and patient survival (Table 3).

We examined the association of 25(OH)D levels with OS and PFS across strata of other prognostic factors. The association of 25(OH)D levels with OS and PFS remained largely unchanged across subgroups, including age, sex, ECOG performance status, prior adjuvant chemotherapy, chemotherapy backbone, assigned treatment arm, primary tumor location, *RAS* mutation status, BMI, physical activity, total vitamin D intake, geographic region of residence, and season of blood collection (all $P_{interaction} \geq 0.11$; Fig. 2).

Discussion

Among 1,041 patients with advanced or metastatic colorectal cancer, we found that 63% of patients were vitamin D deficient and 31% were vitamin D insufficient at baseline. Higher plasma 25(OH)D levels were associated with a significant improvement in OS and PFS. The benefit associated with higher 25(OH)D levels was consistent across most strata of demographic, lifestyle, and pathological characteristics. To our knowledge, this was the largest study of the association between circulating 25(OH)D levels and survival among patients with advanced or metastatic colorectal cancer when it was presented at the Annual Meeting of the American Society of Clinical Oncology in 2015 (22).

The high prevalence of vitamin D deficiency among patients with advanced or metastatic colorectal cancer is consistent with—and indeed more pronounced than—the trend in vitamin D status in the general US population. In the National Health and Nutrition Examination Survey (NHANES) between 2001 and 2004, the mean serum 25(OH)D level was 24 ng/mL among 13,369 participants, indicating a remarkable decrease from the third NHANES (1988–1994), when the mean 25(OH)D level was 30 ng/mL (1). The major causes of this progressive decrease in vitamin D status include avoidance of sun exposure for skin cancer prevention in conjunction with increased use of sunscreen, decreased levels of physical activity, increased percentage of workforce being indoors, and the rising obesity epidemic in the US population. Compared with the general population, our participants with advanced or metastatic colorectal cancer had particularly low levels of 25(OH)D with a mean of 17.7 ng/mL, which was consistent with previous studies (6, 7). We noted that patients with *RAS* mutant tumors had lower 25(OH)D levels than those with *RAS* wild-type tumors. Preclinical studies suggest that *KRAS* mutation could modulate vitamin D activity through the down-regulation of vitamin D receptor (VDR; ref. 23) or resistance to growth inhibition by calcitriol [$1,25(OH)_2D$] (24, 25), the hormonally active form of vitamin D. Although we found no

Table 1. Baseline characteristics by quintile of plasma 25(OH)D

Characteristic	Quintile of plasma 25(OH)D					P
	1 ($n = 208$)	2 ($n = 209$)	3 ($n = 207$)	4 ($n = 209$)	5 ($n = 208$)	
25(OH)D (ng/mL), mean (SD)	7.7 (2.2)	13.4 (1.3)	17.3 (1.1)	21.5 (1.4)	28.8 (5.0)	<0.0001[a]
Age (y), mean (SD)	57.6 (10.6)	59.1 (11.8)	59.6 (12.3)	60.3 (11.3)	60.5 (12.8)	0.09[a]
Sex, No. (%)						0.003[b]
Female	108 (52)	75 (36)	87 (42)	74 (35)	93 (45)	
Male	100 (48)	134 (64)	120 (58)	135 (65)	115 (55)	
Race/ethnicity, No. (%)						<0.0001[c]
White	149 (72)	177 (85)	184 (89)	186 (89)	199 (96)	
Black	52 (25)	25 (12)	12 (6)	15 (7)	5 (2)	
Other	6 (3)	5 (2)	8 (4)	6 (3)	3 (1)	
Unknown	1 (0)	2 (1)	3 (1)	2 (1)	1 (0)	
ECOG performance status[d], No. (%)						0.0004[c]
0	102 (49)	133 (64)	119 (57)	132 (63)	145 (70)	
1	105 (50)	76 (36)	88 (43)	77 (37)	63 (30)	
2	1 (0)	0	0	0	0	
Prior adjuvant chemotherapy, No. (%)						0.22[b]
No	186 (89)	183 (88)	183 (88)	174 (83)	174 (84)	
Yes	22 (11)	26 (12)	24 (12)	35 (17)	34 (16)	
Chemotherapy backbone, No. (%)						0.96[b]
mFOLFOX6	158 (76)	159 (76)	162 (78)	158 (76)	161 (77)	
FOLFIRI	50 (24)	50 (24)	45 (22)	51 (24)	47 (23)	
Assigned treatment arm, No. (%)						0.38[b]
Bevacizumab	86 (41)	74 (35)	79 (38)	87 (42)	94 (45)	
Cetuximab	90 (43)	91 (44)	84 (41)	76 (36)	77 (37)	
Bevacizumab + cetuximab	32 (15)	44 (21)	44 (21)	46 (22)	37 (18)	
Disease extent						0.89[c]
Locally advanced	3 (1)	4 (2)	2 (1)	5 (2)	2 (1)	
Metastatic	205 (99)	204 (98)	205 (99)	203 (97)	205 (99)	
Missing	0	1 (0)	0	1 (0)	1 (0)	
Primary tumor location, No. (%)						0.48[b]
Left (splenic flexure, descending colon, sigmoid, rectum)	121 (58)	123 (59)	132 (64)	114 (55)	120 (58)	
Right (cecum, ascending colon, hepatic flexure)	63 (30)	56 (27)	47 (23)	71 (34)	61 (29)	
Transverse	14 (7)	13 (6)	15 (7)	16 (8)	13 (6)	
Multiple	0	2 (1)	3 (1)	0	1 (0)	
Unknown	10 (5)	15 (7)	10 (5)	8 (4)	13 (6)	
RAS mutation status, No. (%)						0.01[b]
Wild-type	68 (33)	65 (31)	55 (27)	80 (38)	77 (37)	
Mutant	62 (30)	62 (30)	80 (39)	60 (29)	44 (21)	
Unknown	78 (37)	82 (39)	72 (35)	69 (33)	87 (42)	
Body mass index (kg/m^2), mean (SD)	29.4 (7.1)	28.6 (6.4)	28.0 (5.7)	28.2 (6.0)	26.8 (5.6)	0.0008[a]
Physical activity (MET-h/week), mean (SD)	7.6 (16.3)	11.9 (18.9)	9.1 (15.2)	11.3 (19.2)	11.7 (18.6)	0.15[a]
Dietary vitamin D intake, energy-adjusted (IU/day), mean (SD)	203 (116)	211 (119)	214 (109)	225 (118)	235 (130)	0.15[a]
Total vitamin D intake[e], energy-adjusted (IU/day), mean (SD)	261 (218)	356 (257)	413 (277)	519 (332)	556 (357)	<0.0001[a]
Total calcium intake[e], energy-adjusted (mg/day), mean (SD)	997 (503)	1148 (550)	1207 (534)	1241 (533)	1368 (637)	<0.0001[a]
Season of blood collection, No. (%)						0.03[b]
Summer (June, July, August)	35 (17)	43 (21)	40 (19)	56 (27)	59 (28)	
Fall (September, October, November)	33 (16)	42 (20)	40 (19)	39 (19)	47 (23)	
Winter (December, January, February)	43 (21)	49 (23)	43 (21)	50 (24)	34 (16)	
Spring (March, April, May)	97 (47)	74 (35)	84 (41)	64 (31)	68 (33)	
Unknown	0	1 (0)	0	0	0	
Geographic region of residence[f], No. (%)						0.21[c]
Southern US	87 (42)	77 (37)	85 (41)	99 (47)	87 (42)	
Midwestern/western US	81 (39)	97 (46)	82 (40)	65 (31)	85 (41)	
Northeastern US	33 (16)	33 (16)	34 (16)	42 (20)	31 (15)	
Canada	7 (3)	2 (1)	5 (2)	2 (1)	5 (2)	
Unknown	0	0	1 (0)	1 (0)	0	

Abbreviation: MET, metabolic equivalent.

[a]Calculated using analysis of variance test.

[b]Calculated using Chi-squared test.

[c]Calculated using Fisher exact test.

[d]Grade 0 = fully active, able to carry on all pre-disease performance without restriction; grade 1 = restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work; grade 2 = ambulatory and capable of all selfcare but unable to carry out any work activities, up and about more than 50% of waking hours.

[e]Including intake from foods and supplements.

[f]Southern US includes AL, AR, CA, CO, DE, FL, GA, HI, KY, LA, MD, MS, NV, NC, OK, SC, TN, TX, UT, VA, and WV; midwestern/western US includes ID, IL, IN, IA, KS, MI, MN, MO, MT, NE, ND, OH, OR, SD, WA, and WI; northeastern US includes AK, CT, ME, MA, NH, NJ, NY, PA, RI, and VT; Canada includes ON and SK.

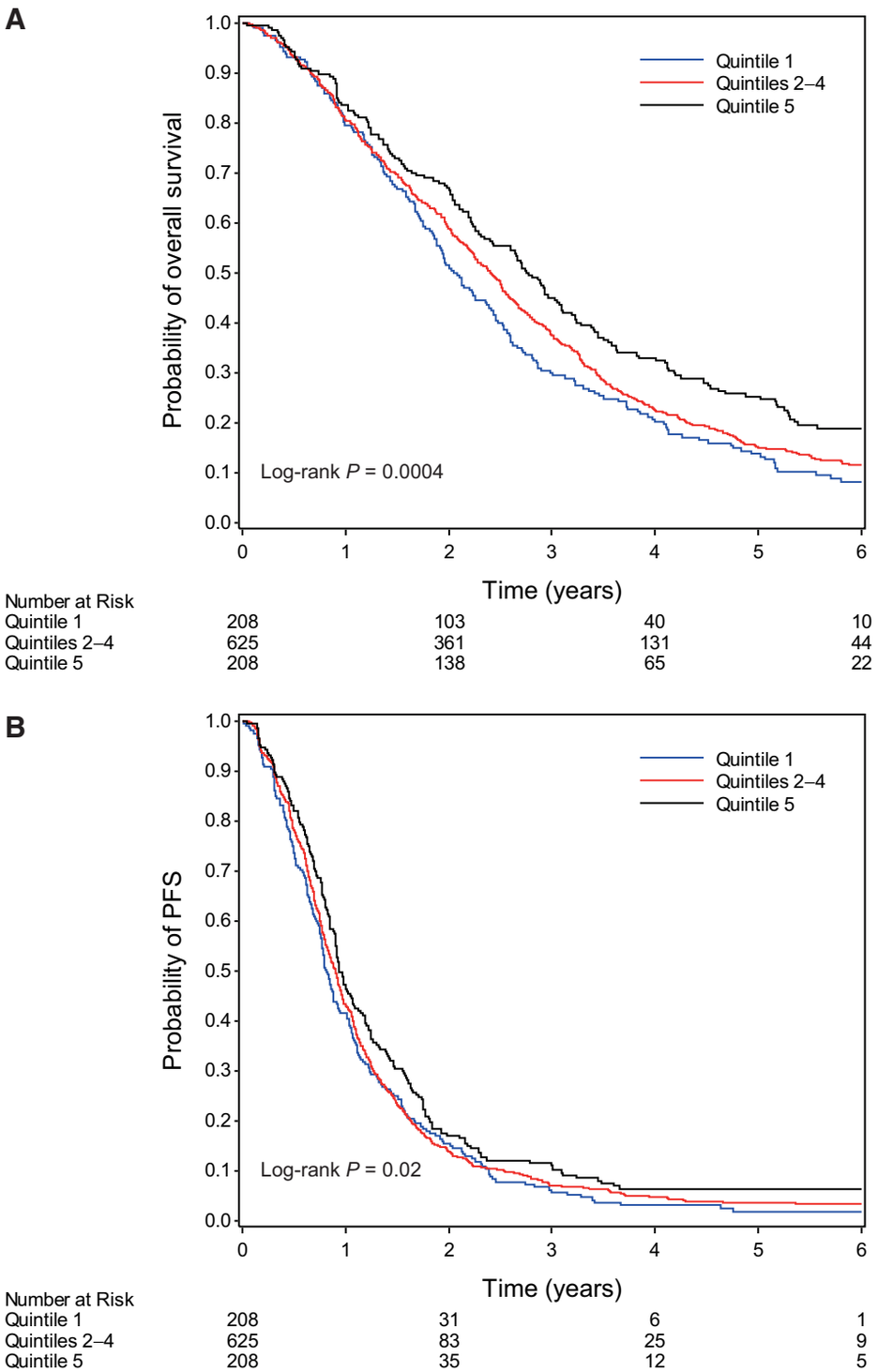

Figure 1.
Kaplan–Meier curves for (**A**) overall survival and (**B**) PFS according to quintile of plasma 25(OH)D. Patients in quintiles 2 to 4 were combined for ease of graphic viewing.

interaction between 25(OH)D levels and *RAS* mutation on patient survival, further research is warranted to investigate the underlying mechanisms.

The observed association between 25(OH)D levels and survival among patients with advanced or metastatic colorectal cancer is consistent with prior findings. We previously reported that higher prediagnostic plasma 25(OH)D levels were associated with improved OS in 304 patients with all stages of colorectal cancer from two prospective cohort studies (10). In another study of 515 patients with metastatic colorectal cancer nested within the North Central Cancer Treatment Group trial N9741, we noted no association between plasma 25(OH)D levels and OS in the entire

Table 2. HRs for OS and PFS by quintile of plasma 25(OH)D

	Quintile of plasma 25(OH)D					
	1	2	3	4	5	P_{trend}[a]
Median (range) (ng/mL)	8.0 (2.2–10.8)	13.6 (10.9–15.4)	17.2 (15.4–19.2)	21.3 (19.3–24.0)	27.5 (24.1–72.7)	
OS						
No. of events/No. of patients	185/208	184/209	177/207	177/209	170/208	
Median OS (95% CI), mo	25 (22–29)	30 (27–33)	28 (24–31)	27 (24–32)	33 (28–37)	
Unadjusted HR (95% CI)	1	0.86 (0.70–1.05)	0.87 (0.71–1.07)	0.84 (0.68–1.03)	0.70 (0.57–0.86)	0.001
Multivariable-adjusted HR (95% CI)[b]	1	0.82 (0.66–1.02)	0.80 (0.64–0.99)	0.80 (0.65–1.00)	0.66 (0.53–0.83)	0.0009
PFS						
No. of events/No. of patients	200/208	203/209	194/207	196/209	194/208	
Median PFS (95% CI), mo	10 (9-11)	10 (9–11)	10 (9–12)	11 (10–13)	11 (11–13)	
Unadjusted HR (95% CI)	1	1.02 (0.84–1.24)	0.91 (0.74–1.10)	0.89 (0.73–1.08)	0.80 (0.65–0.97)	0.01
Multivariable-adjusted HR (95% CI)[b]	1	1.00 (0.82–1.23)	0.89 (0.72–1.09)	0.91 (0.73–1.12)	0.81 (0.66–1.00)	0.03

[a]Calculated by entering quintile-specific median values for 25(OH)D as a continuous variable.

[b]Adjusted for age (continuous), sex (female, male), race (white, black, other, unknown), Eastern Cooperative Oncology Group performance status (0, 1, 2), prior adjuvant chemotherapy (yes, no), chemotherapy backbone (leucovorin, fluorouracil, and oxaliplatin; leucovorin, fluorouracil, and irinotecan), assigned treatment arm (bevacizumab, cetuximab, bevacizumab + cetuximab), *RAS* mutation status (wild-type, mutant, unknown), body mass index (continuous), physical activity (continuous), season of blood collection (summer, fall, winter, spring, unknown), and geographic region of residence (southern US, midwestern/western US, northeastern US, Canada, unknown).

population but a benefit of higher 25(OH)D levels on OS among patients receiving FOLFOX (6). Recently, a meta-analysis of 11 studies, including the abovementioned two, with a total of 7,718 patients with CRC found a robust association of higher circulating 25(OH)D levels with improved overall and colorectal cancer-specific survival (26). In addition, the promising results of the current study led to a randomized double-blind phase II trial, SUNSHINE, of 139 patients with *KRAS* wild-type advanced or metastatic colorectal cancer, to test whether vitamin D supplementation to raise plasma 25(OH)D levels can improve outcomes in these patients. This trial was recently published, showing that patients randomized to high-dose vitamin D supplementation (4,000 IU/day) had sufficiently increased 25(OH)D levels and improved PFS compared with those receiving lose-dose vitamin D supplementation (400 IU/day; ref. 7).

Abundant preclinical evidence supports the hypothesis that vitamin D may possess antineoplastic activity against colorectal cancer. VDR and 1-α-hydroxylase, which converts 25(OH)D into $1,25(OH)_2D$, are present in colon cancer cells (27–29). The binding of VDR by $1,25(OH)_2D$ promotes differentiation (30, 31), activates apoptotic pathways (32), and inhibits angiogenesis (33, 34), proliferation (35), and metastasis (36) of colon cancer. In the genetically engineered mouse model of intestinal carcinogenesis (APC^{min}), tumor burden was significantly increased by inactivation of the *VDR* gene (37) and decreased by treatment with vitamin D or its synthetic analogue (38). Other mechanisms through which vitamin D may influence colorectal carcinogenesis include modulation of cellular immunity and systematic inflammation (39, 40).

This study has several strengths. The patient population was large and drawn from a rigorously conducted, multicenter NCTN phase III randomized clinical trial. All patients had pathologically proven advanced or metastatic colorectal cancer at study entry, with standardized treatment and follow-up care, as well as regular examinations to prospectively record the date and nature of cancer progression. Extensive and detailed information on lifestyle and disease characteristics was prospectively collected, so we were able to accurately adjust for potential confounders and assess their interactions with 25(OH)D levels on survival.

Nonetheless, several potential limitations warrant discussion. Because 25(OH)D levels were only measured once at study entry, the impact of changes in these levels on survival could not be studied. It is possible that lower baseline 25(OH)D levels are a surrogate for greater burden of cancer, inadequate nutrition, or limited physical activity from illness, all of which are associated with worse survival. We adjusted for these factors in multivariable analyses and continued to see a significant independent effect of higher vitamin D status on improved survival, and more

Table 3. HRs for OS and PFS by clinical category of plasma 25(OH)D

	Plasma 25(OH)D level		
	<10 ng/mL	10 to <20 ng/mL	≥20 ng/mL
OS			
No. of events/No. of patients	135/152	426/491	332/398
Median OS (95% CI), months	23 (20–26)	29 (27–31)	31 (27–34)
Unadjusted HR (95% CI)	1	0.82 (0.67–0.99)	0.72 (0.59–0.88)
Multivariable-adjusted HR (95% CI)[a]	1	0.78 (0.64–0.96)	0.70 (0.56–0.86)
PFS			
No. of events/No. of patients	146/152	466/491	375/398
Median PFS (95% CI), months	9 (9–11)	10 (9–11)	11 (11–13)
Unadjusted HR (95% CI)	1	0.88 (0.73–1.06)	0.80 (0.66–0.96)
Multivariable-adjusted HR (95% CI)[a]	1	0.87 (0.71–1.06)	0.81 (0.66–1.00)

[a]Adjusted for age (continuous), sex (female, male), race (white, black, other, unknown), Eastern Cooperative Oncology Group performance status (0, 1, 2), prior adjuvant chemotherapy (yes, no), chemotherapy backbone (leucovorin, fluorouracil, and oxaliplatin; leucovorin, fluorouracil, and irinotecan), assigned treatment arm (bevacizumab, cetuximab, bevacizumab + cetuximab), *RAS* mutation status (wild-type, mutant, unknown), body mass index (continuous), physical activity (continuous), season of blood collection (summer, fall, winter, spring, unknown), and geographic region of residence (southern US, midwestern/western US, northeastern US, Canada, unknown).

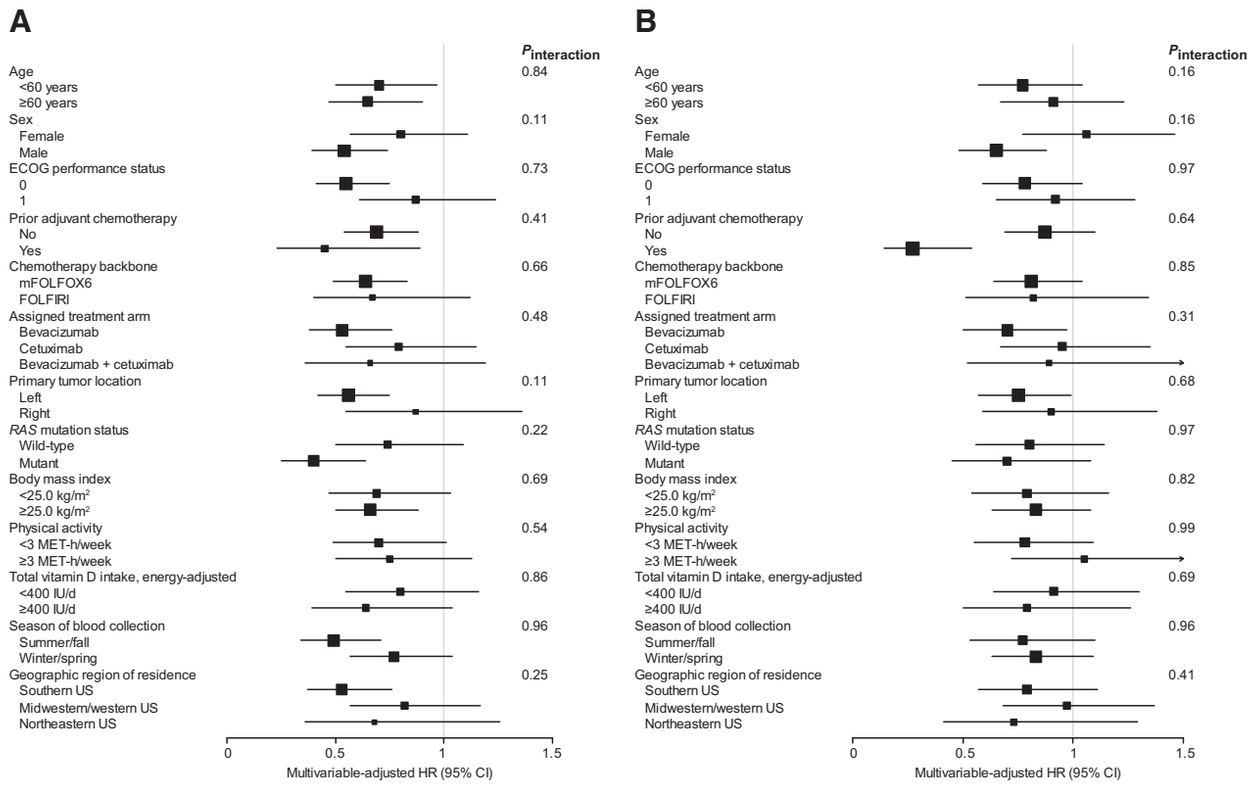

Figure 2.

Multivariable-adjusted HRs and 95% CIs for (**A**) overall survival and (**B**) PFS, comparing the highest to lowest quintile of plasma 25(OH)D, across strata of potential effect modifiers. Adjusted for age (continuous), sex (female, male), race (white, black, other, unknown), ECOG performance status (0, 1, 2), prior adjuvant chemotherapy (yes, no), chemotherapy backbone (mFOLFOX6; FOLFIRI), assigned treatment arm (bevacizumab, cetuximab, bevacizumab + cetuximab), *RAS* mutation status (wild-type, mutant, unknown), body mass index (continuous), physical activity (continuous), season of blood collection (summer, fall, winter, spring, unknown), and geographic region of residence (southern US, midwestern/western US, northeastern US, Canada, unknown), excluding the stratification variable. MET, metabolic equivalent.

importantly, our SUNSHINE randomized phase II trial (7) supports causality in the relationship between vitamin D and colorectal cancer survival. Finally, patients who enroll on clinical trials may not be representative of the population at large. Although CALGB/SWOG 80405 (Alliance) was conducted within the NCTN, which is composed of both academic and community hospitals throughout North America, our participants were predominantly individuals of European descent, and additional studies in other populations are warranted.

In conclusion, we observed a particularly high prevalence of vitamin D deficiency among patients with advanced or metastatic colorectal cancer. In light of our findings that higher 25(OH)D levels are associated with improved OS and PFS, randomized trials are warranted to assess the benefit of vitamin D supplementation in patients with colorectal cancer. These findings, followed by the promising results of our SUNSHINE randomized phase II trial (7), have now paved the way for an Alliance-led randomized phase III trial of vitamin D supplementation in combination with standard chemotherapy plus biologic therapy among previously untreated patients with metastatic colorectal cancer (SOLARIS, Protocol A021703) to confirm causality. Correlative research using biospecimens from these clinical trial cohorts are also warranted to further elucidate underlying mechanisms of action.

Disclosure of Potential Conflicts of Interest

B.H. O'Neil is an employee/paid consultant for Lilly. H-J. Lenz reports receiving commercial research grants from Bayer and reports receiving speakers bureau honoraria from Bayer, Merck KG and Roche. C.S. Fuchs holds ownership interest (including patents) in Cytomx Therapeutics and Entrinsic Health, and is an advisory board member/unpaid consultant for Agios, Bain Capital, Bayer, Celgene, Dicerna, Five Prime Therapeutics, Gilead Sciences, Eli Lilly, Entrinsic Health, Genentech, KEW, Merck, Merrimack Pharma, Pfizer, Sanofi, Taiho, Unum Therapeutics, and CytomX Therapeutics. J.A. Meyerhardt is an employee/paid consultant for COTA and Ignyta, and reports receiving other remuneration from Taiho (NCCN grant committee for Taiho Grant Support). K. Ng is an employee/paid consultant for Seattle Genetics, Bayer, Lilly, Genentech, and Tarrex, and reports receiving commercial research grants from Pharmavite, LLC, Genentech, Gilead, Tarrex, Trovagene, and Celgene. No potential conflicts of interest were disclosed by the other authors.

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