Vitamin D Status in Patients With Stage IV Colorectal Cancer: Findings From Intergroup Trial N9741

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A B S T R A C T

Purpose
Previous studies have suggested that higher plasma 25-hydroxyvitamin D_3 [25(OH)D] levels are associated with decreased colorectal cancer risk and improved survival, but the prevalence of vitamin D deficiency in advanced colorectal cancer and its influence on outcomes are unknown.

Patients and Methods
We prospectively measured plasma 25(OH)D levels in 515 patients with stage IV colorectal cancer participating in a randomized trial of chemotherapy. Vitamin D deficiency was defined as 25(OH)D lower than 20 ng/mL, insufficiency as 20 to 29 ng/mL, and sufficiency as $\geq$ 30 ng/mL. We examined the association between baseline 25(OH)D level and selected patient characteristics. Cox proportional hazards models were used to calculate hazard ratios (HR) for death, disease progression, and tumor response, adjusted for prognostic factors.

Results
Among 515 eligible patients, 50% of the study population was vitamin D deficient, and 82% were vitamin D insufficient. Plasma 25(OH)D levels were lower in black patients compared to white patients and patients of other race (median, 10.7 *v* 21.1 *v* 19.3 ng/mL, respectively; $P < .001$), and females compared to males (median, 18.3 *v* 21.7 ng/mL, respectively; $P = .0005$). Baseline plasma 25(OH)D levels were not associated with patient outcome, although given the distribution of plasma levels in this cohort, statistical power for survival analyses were limited.

Conclusion
Vitamin D deficiency is highly prevalent among patients with stage IV colorectal cancer receiving first-line chemotherapy, particularly in black and female patients.

INTRODUCTION

Vitamin D insufficiency is highly prevalent in the United States. Recent data indicate that 77% of Americans have plasma 25-hydroxyvitamin D_3 [25(OH)D] levels lower than 30 ng/mL, the threshold required for adequate bone health.[1] These low levels of vitamin D are concerning in light of increasing evidence that vitamin D may have health benefits beyond skeletal outcomes, including reducing the risk of and mortality from colorectal cancer.[2,3]

Scientific observations support a role for vitamin D in colorectal cancer pathogenesis, with almost ubiquitous expression in colon cancer cells of vitamin D receptors (VDR)[4,5] and 1-α-hydroxylase,[6] which converts plasma 25(OH)D into 1,25-dihyroxycholecalciferol [1,25$(OH)_2$D]. Binding of VDR by 1,25$(OH)_2$D leads to induction of differentiation and apoptosis,[7,8] and inhibition of proliferation,[9] angiogenesis,[10,11] and metastatic potential.[12,13] Vitamin D also interacts with other pathways involved in colorectal cancer, including the insulin-like growth factor (IGF) pathway. For example, 1,25$(OH)_2$D influences mitogen-activated protein kinase (MAPK) –extracellular signal-regulated kinase (ERK) signaling through effects on IGF-1, and also induces apoptosis through the IGF receptor-1 (IGFR-1) –phosphatidylinositol 3-kinase (PI3K) –Akt signaling pathway.[14]

Although two studies of patients with established colorectal cancer found that higher 25(OH)D levels were significantly associated with improved survival,[15,16] the vitamin D status of patients with metastatic colorectal cancer specifically has not been studied. We therefore examined a cohort of patients with stage IV colorectal cancer enrolled in a large, completed, Intergroup-sponsored clinical trial of palliative chemotherapy, and sought to determine

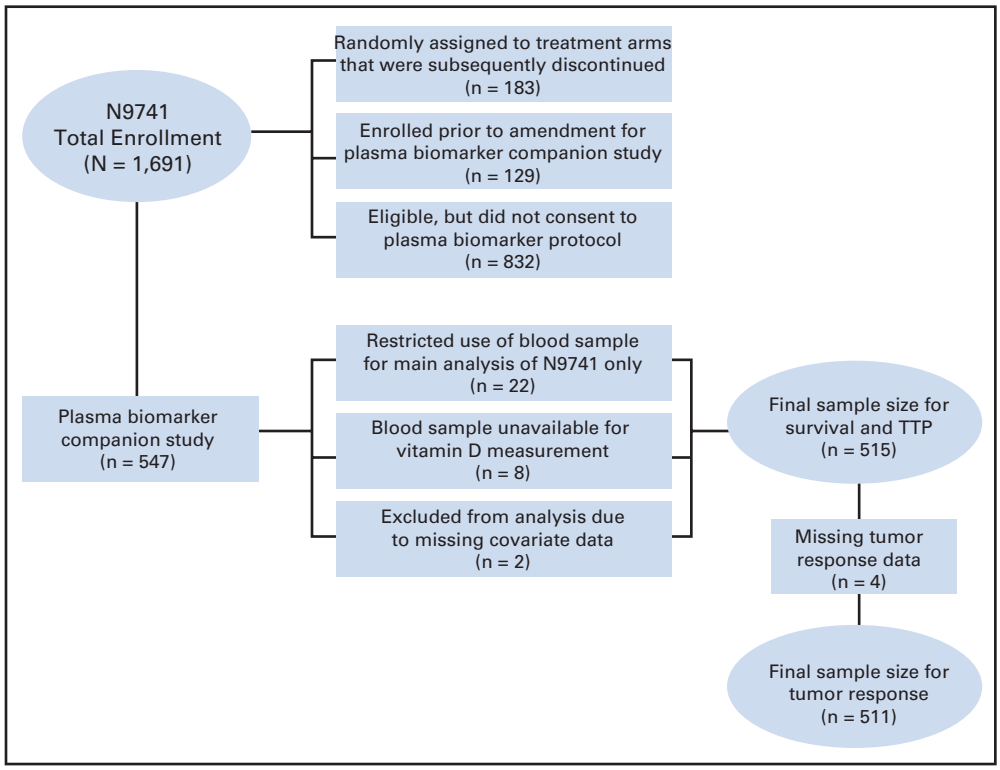

Fig 1. Derivation of cohort size. N9741, Intergroup trial N9741; TTP, time to progression.

the prevalence of vitamin D deficiency and whether vitamin D status was associated with improved treatment outcomes.

PATIENTS AND METHODS

Study Population

Patients included in this study were drawn from a national, Intergroup trial of chemotherapy for metastatic colorectal cancer (N9741). Patients were randomly assigned between October 1998 and April 2001 to receive bolus fluorouracil, leucovorin, and irinotecan (IFL); infusional fluorouracil, leucovorin, and oxaliplatin (FOLFOX); or irinotecan and oxaliplatin (IROX). Full details and results of the treatment trial have previously been published.[17] Briefly, patients were required to have histologically proven unresectable colorectal adenocarcinoma, Eastern Cooperative Oncology Group performance status of 2 or lower, and adequate renal, liver and bone marrow function. Exclusion criteria included prior therapy for advanced disease, baseline peripheral neuropathy, or CNS disease, uncontrolled or severe comorbid illnesses, and baseline $\geq$ 3 loose stools per day. The protocol was reviewed and approved by the institutional review board of each participating institution. Patients signed informed consent for participation in the trial and were given the option of inclusion in a companion study of plasma for future research.

In total, 1,379 patients were enrolled in N9741 after the incorporation of an amendment to collect blood samples for companion biomarker studies. Of these, 547 patients provided blood samples, with 515 available for this analysis. Figure 1 illustrates the derivation of the final cohort size and reasons for exclusion at different time points. Among the 515 eligible patients, 239 (46%) had a confirmed tumor response. Since study enrollment, 440 patients (85%) have progressed and 475 (92%) have died. The median time of follow-up among living patients was 5.1 years (10th and 90th percentiles: 3.3 and 6.1 years, respectively). In a previous report, we compared baseline characteristics of the overall cohort of patients who enrolled in the treatment trial with the subset participating in the biomarker studies, and did not detect any appreciable differences between the two groups.[18] Patients who did and did not provide blood samples experienced similar overall survival (median 18.1 and 17.0 months, respectively).

Response and Progression Criteria

Study enrollment required at least one measurable lesion ($\geq$ 2 cm in diameter) or disease that could be serially evaluated to establish whether the disease was getting better or worse (evaluable disease). Objective response to chemotherapy was calculated among patients evaluable for response (n = 511), whereas time to progression and overall survival were assessed among all subjects (n = 515). Partial response required at least a 50% reduction in the sum of the products of the longest perpendicular diameters of all measurable lesions. Regression required documented tumor reduction in evaluable patients who did not have disease that met the guidelines for measurable disease. Disease progression required $\geq$ 25% increase in measurable tumor or an increase in tumor size in patients whose lesions did not meet criteria for measurable disease. After partial response, tumor measurements exceeding 50% of the maximal extent of a previously observed reduction constituted progression. Any new lesion constituted progression. Patients who did not meet the definitions of response or progression were classified as having stable disease.

Time to progression (TTP) was calculated from study entry to disease progression, regardless of the patient's treatment status. Deaths occurring within 30 days of treatment discontinuation were considered progressions. Survival was calculated from enrollment to death or last contact. Without contradictory data, patients who died or were lost to follow-up were assumed to have progressed at the time they were last documented to be progression free.

Plasma Biomarker Assessment

Blood samples were collected on study registration and sent to the Mayo Clinic central laboratory for clinical trials (Rochester, MN). Whole-blood samples for this analysis were cooled and sent by overnight delivery to Heartland Assays Inc (Ames, IA), and 25(OH)D concentrations measured by radioimmunoassay.[19] Masked quality control samples were interspersed among the case samples, and all laboratory personnel were blinded to patient outcome. The mean coefficient of variation of the assay was 8%.

Table 1. Baseline Patient Characteristics (n = 515)

Characteristic	No.	%
Median plasma 25(OH)D, ng/mL	20.0	
Range	2.3-75.4	
Median age, years	61	
Range	26-85	
Sex		
Female	209	41
Male	306	59
Race or ethnicity		
White	441	86
Black	40	8
Hispanic	16	3
Asian, Native Hawaiian, other Pacific Islander	10	2
Other	3	< 1
Unknown/missing	5	1
ECOG performance status*		
0-1	492	96
2	22	4
Missing	1	< 1
Median body mass index, kg/m^2†	26.1	
Range	15.4-49.1	
Prior adjuvant chemotherapy		
Yes	79	15
No	435	84
Missing	1	1
Median No. of metastatic sites	2	
Range	1-6	
Liver-only metastasis		
Yes	132	26
No	383	74
One metastatic site, not liver		
Yes	40	8
No	475	92
Multiple metastatic sites, none liver		
Yes	63	12
No	452	88
Treatment arm		
IFL	112	22
FOLFOX	299	58
IROX	104	20
Season of blood collection‡		
Summer	140	27
Autumn	83	16
Winter	141	27
Spring	151	30
Geographic region of registering site§		
Northeastern US	73	14
Midwestern US	238	46
Southern US	80	16
Western US	58	11
Puerto Rico	7	2
Canada	48	9
Missing	11	2

Abbreviations: 25(OH)D, 25-hydroxyvitamin D_3; ECOG, Eastern Cooperative Oncology Group; IFL, irinotecan, bolus fluorouracil, leucovorin; FOLFOX, fluorouracil, leucovorin, oxaliplatin; IROX, irinotecan, oxaliplatin; US, United States.

*A performance status of 0 indicates a patient was fully active; 1, restricted in physically strenuous activity but ambulatory and able to carry out light work; 2, ambulatory and capable of all self-care but unable to carry out any work activities and up and about more than 50% of waking hours. Performance status was based on patient status at initiation of chemotherapy (entry into treatment trial).

†Body mass index is calculated as weight in kilograms divided by height in meters squared. Body mass index was based on patient status at initiation of chemotherapy (entry into treatment trial).

‡Seasons defined as: Summer = June, July, August; Autumn = September, October, November; Winter = December, January, February; Spring = March, April, May.

§Geographic regions defined according to the US Census Bureau: Northeast = CT, ME, MA, NH, NJ, NY, PA, RI, VT; Midwest = IL, IN, IA, KS, MI, MN, MO, NE, ND, OH, SD, WI; South = AL, DE, FL, GA, KY, LA, MD, NC, OK, SC, TN, TX, VA, WV; West = AZ, CA, CO, HI, ID, MT, NV, NM, OR, UT, WA, WY.

IGF-1, IGF-2, IGF binding protein (IGFBP) -3, and C-peptide levels were assayed in the laboratory of Michael Pollak, MD (Jewish General Hospital, McGill University, Montreal) using enzyme-linked immunosorbent assays, as previously described.[20]

Statistical Analyses

Descriptive analyses were performed for demographic and clinical characteristics. Vitamin D deficiency was prespecified as plasma 25(OH)D lower than 20 ng/mL, insufficiency as 20 to 29 ng/mL, and sufficiency as ≥ 30 ng/mL.[21] The association between vitamin D levels and patient characteristics was described with percentages and medians and compared using the Wilcoxon rank sum test and Kruskal-Wallis test. Correlations between plasma 25(OH)D and liver function tests and plasma IGF factors were calculated using Spearman correlation coefficients.

The primary statistical analysis used to evaluate the association between vitamin D status and patient outcome was the two-tailed linear test for trend, using plasma 25(OH)D as a continuous variable,[22] because the plasma 25(OH)D levels were generally consistent with a normal distribution, and in order to avoid the possibility of selecting cut points with maximal *P* values. To facilitate the display of our results, plasma 25(OH)D levels were defined in quartiles. Logistic regression was used to calculate the relative risks for objective response adjusted for other patient and disease characteristics. Cox proportional hazards modeling was used to calculate multivariate hazard ratios (HR) and 95% CI.[23] Collinearity of the covariates was assessed through correlation indices and the condition matrix. The highest Pearson correlation between any two variables was 0.195, and the condition index of the multivariate model was 25.3, suggesting that collinearity is not an issue of concern.

In secondary exploratory analyses, tests of interaction between plasma 25(OH)D and potential effect modifiers were assessed by entering in the model the cross product term of 25(OH)D as a continuous variable with the covariate as a continuous or binary variable. Analyses stratified by the dichotomized covariate were performed using the initial quartile cutoffs that were calculated for the entire cohort. Two-sided *P* values lower than .05 were used to denote statistical significance. All analyses used SAS software, version 9.1 (SAS Institute Inc, Cary, NC).

RESULTS

Distribution of Vitamin D Levels

Baseline characteristics of the 515 included patients are presented in Table 1. The median age was 61 years (range, 26 to 85), with 59% males and 41% females. The vast majority of patients were white (86%). The median plasma 25(OH)D level in the entire cohort was 20.0 ng/mL (range, 2.3 to 75.4 ng/mL) and the mean was 21.0 ng/mL (SE of mean, 0.4 ng/mL). At the start of chemotherapy, 50% of the study population were vitamin D deficient (< 20 ng/mL), and 82% were vitamin D insufficient (< 30 ng/mL). Only 10% of patients had plasma 25(OH)D levels ≥ 33 ng/mL, the threshold believed to be required for a potential protective effect on colorectal cancer risk.[24] Figure 2 summarizes the distribution of plasma 25(OH)D levels in the study cohort.

Association of Vitamin D Levels With Patient Characteristics

Plasma 25(OH)D levels were significantly lower in females compared to males (median, 18.3 *v* 21.7 ng/mL, respectively; $P = .0005$; Table 2). Plasma 25(OH)D levels were also significantly lower in black patients compared to white patients and patients of other or unknown race (median, 10.7, 21.1, and 19.3 ng/mL, respectively; $P < .001$). Vitamin D deficiency was more common among black patients (80%) compared with white patients (47%) and those of other or unknown race (56%; $P = .002$). Moreover, only 4% of blacks, 11% of whites, and 9% of subjects of other or unknown race demonstrated plasma 25(OH)D levels ≥ 33 ng/mL. As expected, plasma 25(OH)D levels varied significantly by season of blood collection ($P < .001$). Plasma 25(OH)D concentration was not significantly associated with patient age, performance status, body mass index, chemotherapy arm, geographical region of registering site, number of metastatic sites, or presence of liver metastases. Moreover, plasma 25(OH)D levels were not significantly correlated with total bilirubin ($r = -0.03$), alkaline phosphatase ($r = -0.01$), or aspartate aminotransferase (AST or SGOT; $r = 0.03$). We also explored the correlation between plasma 25(OH)D and levels of plasma IGF factors, since preclinical data suggest that the two pathways may interact.[25,26] No significant correlations were detected between plasma 25(OH)D and IGF-1 ($r = 0.05$), IGF-2 ($r = 0.04$), IGFBP-3 ($r = -0.002$), or C-peptide ($r = -0.04$).

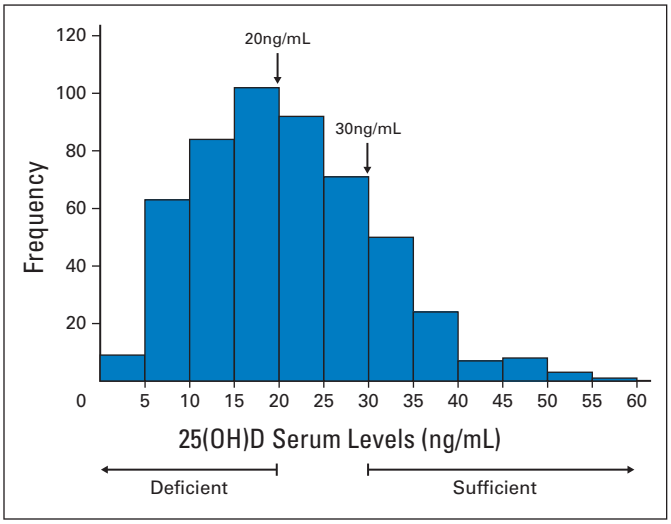

Fig 2. Distribution of serum 25-hydroxyvitamin D [25(OH)D] levels in the study cohort (n = 515).

Impact of Vitamin D Levels on Patient Outcome

Despite the finding that only 10% of our cohort demonstrated plasma 25(OH)D levels ≥ 33 ng/mL at baseline, we still examined the association between 25(OH)D levels and patient outcome. We did not detect a significant relationship between higher levels of 25(OH)D and overall survival, TTP, or tumor response (Table 3). Compared to patients in the lowest quartile, those with plasma 25(OH)D levels in the highest quartile had a multivariate HR for death of 0.94 (95% CI, 0.72 to 1.23; *P* trend = .55) and a multivariate HR for disease progression of 1.07 (95% CI, 0.81 to 1.42; *P* trend = .66). Higher vitamin D levels were also not associated with improved tumor response (multivariate RR 1.12 comparing the highest to lowest quartile; 95% CI, 0.67 to 1.89; *P* trend = .67). Our results did not change appreciably when we excluded poor performance status patients and those with body mass index ≤ 23 kg/m^2 (reflecting impaired nutritional status),[20] or when we additionally adjusted for geographic region.

Impact of Vitamin D Levels Across Strata of Other Predictors of Patient Outcome

We examined the influence of plasma 25(OH)D on overall survival across strata of other predictors of cancer outcome (Fig 3). Most subgroups did not show a significant relationship between plasma

Table 2. Relationship of 25(OH)D Levels to Selected Patient Characteristics

Characteristic	No. of Patients	%	25(OH)D (ng/mL) Median	25(OH)D (ng/mL) Range	*P*
Age, years*					.36†
< 61	244	47	20.0	2.3-52.2	
≥ 61	271	53	20.4	4.3-75.4	
Sex					.0005†
Female	209	41	18.3	2.3-55.4	
Male	306	59	21.7	4.5-75.4	
Race or ethnicity					< .001‡
White	441	86	21.1	2.3-75.4	
Black	40	8	10.7	4.3-49.8	
Other/unknown	34	6	19.3	9.0-35.8	
ECOG performance status					.85†
0-1	493	96	21.1	2.3-75.4	
2	22	4	19.3	7.1-32.1	
Body mass index, kg/m^2 *§					.17†
< 26.1	253	49	21.1	2.3-75.4	
≥ 26.1	262	51	18.8	3.9-48.8	
Treatment arm					.51‡
IFL	112	22	18.6	5.1-75.4	
FOLFOX	299	58	21.1	4.3-52.1	
IROX	104	20	19.2	2.3-51.2	
Season of blood collection‖					< .001‡
Summer	140	27	22.6	4.3-49.4	
Autumn	83	16	24.2	4.3-55.4	
Winter	141	27	18.8	4.7-75.4	
Spring	151	30	16.5	2.3-48.8	
Geographic region of registering site¶					.82‡
Northeastern US + Canada	121	24	21.2	4.7-51.2	
Midwestern US	238	47	19.0	2.3-75.4	
Southern US + Puerto Rico	87	17	21.8	3.9-52.1	
Western US	58	12	19.8	4.8-38.2	
No. of metastatic sites*					.63†
< 2	172	33	21.6	4.3-49.8	
≥ 2	343	67	19.6	3.2-75.4	
Liver metastases					.09†
Yes	410	80	19.2	2.3-75.4	
No	103	20	23.0	6.0-49.8	

Abbreviations: 25(OH)D, 25-hydroxyvitamin D_3; ECOG, Eastern Cooperative Oncology Group; IFL, irinotecan, bolus fluorouracil, leucovorin; FOLFOX, fluorouracil, leucovorin, oxaliplatin; IROX, irinotecan, oxaliplatin; US, United States.

*Cut points chosen based on median values.

†*P* calculated using Wilcoxon rank sum test.

‡*P* calculated using Kruskal-Wallis test.

§Body mass index is calculated as weight in kilograms divided by height in meters squared. Body mass index was based on patient status at initiation of chemotherapy (entry into treatment trial).

‖Seasons defined as: Summer = June, July, August; Autumn = September, October, November; Winter = December, January, February; Spring = March, April, May.

¶Geographic regions defined according to the US Census Bureau: Northeast = CT, ME, MA, NH, NJ, NY, PA, RI, VT; Midwest = IL, IN, IA, KS, MI, MN, MO, NE, ND, OH, SD, WI; South = AL, DE, FL, GA, KY, LA, MD, NC, OK, SC, TN, TX, VA, WV; West = AZ, CA, CO, HI, ID, MT, NV, NM, OR, UT, WA, WY.

25(OH)D and survival, consistent with our primary analysis. However, a significant interaction was observed with treatment arm (*P* interaction = .0005 using the likelihood ratio test). Among patients randomly assigned to FOLFOX, higher levels of 25(OH)D were significantly associated with improved survival (adjusted HR 0.64 comparing extreme quartiles; 95% CI, 0.45 to 0.90; *P* trend = .003); but among those randomized to IFL, higher levels of 25(OH)D were detrimental (adjusted HR, 2.41; 95% CI, 1.32 to 4.39; *P* trend = .008). Plasma 25(OH)D was not significantly associated with survival in patients randomly assigned to IROX.

We considered the possibility that the beneficial effect of higher plasma 25(OH)D levels among patients receiving FOLFOX may suggest that, in stage IV disease, an improved outcome associated with higher vitamin D levels is limited to subjects who experience a reduction in tumor burden from chemotherapy. We therefore repeated the subgroup analyses using a landmark analysis that adjusted for patients with a confirmed tumor response at 18 weeks. However, our results remained unchanged, with a persistent benefit of higher plasma 25(OH)D levels seen among patients randomly assigned to FOLFOX.

In an additional subgroup analysis, we observed a trend toward a beneficial impact of higher 25(OH)D levels on survival in patients with IGFBP-3 levels above the median (*P* interaction = .07). Significant interactions were not detected for overall survival between plasma 25(OH)D and IGF-1, IGF-2, and C-peptide.

Table 3. Impact of 25(OH)D on Overall Survival, Time to Progression, and Confirmed Tumor Response

Parameter	Plasma 25(OH)D (ng/mL)* by Quartile				*P* for Trend†
	1	2	3	4	
Overall survival					
No. at risk	128	128	129	130	
No. of events	121	114	118	122	
Age- and season-adjusted HR	Referent	0.86	1.00	0.99	.81
95% CI		0.66 to 1.11	0.78 to 1.30	0.77 to 1.29	
Multivariate HR‡	Referent	0.78	1.13	0.94	.55
95% CI		0.60 to 1.02	0.87 to 1.47	0.72 to 1.23	
Time to progression					
No. at risk	128	128	129	130	
No. of events	106	114	110	110	
Age- and season-adjusted HR	Referent	1.17	1.17	1.14	.97
95% CI		0.90 to 1.53	0.89 to 1.53	0.86 to 1.49	
Multivariate HR‡	Referent	1.14	1.23	1.07	.66
95% CI		0.87 to 1.49	0.93 to 1.62	0.81 to 1.42	
Confirmed response					
No. at risk	128	126	128	129	
No. of events	56	60	59	64	
Age- and season-adjusted RR	Referent	1.12	1.05	1.15	.59
95% CI		0.68 to 1.84	0.64 to 1.72	0.70 to 1.91	
Multivariate RR‡	Referent	1.15	0.98	1.12	.67
95% CI		0.70 to 1.91	0.59 to 1.63	0.67 to 1.89	

Abbreviations: 25(OH)D, 25-hydroxyvitamin D_3; HR, hazard ratio; RR, relative risk; IFL, irinotecan, bolus fluorouracil, leucovorin; FOLFOX, fluorouracil, leucovorin, oxaliplatin; IROX, irinotecan, oxaliplatin.

*Quartile 1, 2.3-13.1 ng/mL; quartile 2, 13.2-19.9 ng/mL; quartile 3, 20.0-27.1 ng/mL; quartile 4, 27.2-75.4 ng/mL.

†Calculated by using 25(OH)D as a continuous variable.

‡Adjusted for age (in years as a continuous variable), season of blood collection (summer, autumn, winter, spring), sex (male, female), baseline performance status (0-1, 2), treatment arm (IFL, FOLFOX4, IROX), body mass index (in kg/m^2 as a continuous variable), and metastatic sites (liver only, liver + any other site, single non-liver, multiple non-liver).

DISCUSSION

In this cohort of patients with previously untreated metastatic colorectal cancer, we found a high prevalence of vitamin D deficiency, particularly among black and female patients. Vitamin D insufficiency was seen in 82% of patients and vitamin D deficiency in 50%. Only 10% of patients had plasma 25(OH)D levels $\geq$ 33 ng/mL, the threshold believed to be required for a potential protective effect of vitamin D on colorectal cancer risk.[24] Only 8% of female and 4% of black patients achieved levels $\geq$ 33 ng/mL. Perhaps owing to this skewed distribution of 25(OH)D levels in the cohort, higher plasma 25(OH)D levels were not significantly associated with improved overall survival, TTP, or tumor response. To our knowledge, this is the first study to prospectively characterize the vitamin D status of patients with stage IV colorectal cancer.

Our findings are consistent with a recent analysis of the National Health and Nutrition Examination Survey (NHANES), which found a low mean plasma 25(OH)D level of 24 ng/mL among 13,369 participants between 2001 and 2004.[1] This represented a marked decrease from NHANES III (1988 to 1994), when the mean 25(OH)D level was 30 ng/mL. Potential explanations for the rise in vitamin D insufficiency include increasing use of sunscreen for skin cancer prevention, decreased outdoor activity, and the rising prevalence of obesity. Rates of vitamin D insufficiency in our cohort were even higher than NHANES, possibly reflecting an inverse association of plasma 25(OH)D with colorectal cancer incidence and/or dietary and lifestyle changes after cancer diagnosis. A recent study of patients with stage I to III breast cancer similarly described a high prevalence of vitamin D deficiency and found that supplementation using standard dosage guidelines (400 U daily) was inadequate for raising levels into the sufficient range.[27] The greater prevalence of vitamin D deficiency among black patients in our cohort is consistent with other studies,[1,27,28] and is largely explained by lower vitamin D synthesis in skin due to greater melanin content.[29] Melanin effectively filters ultraviolet B radiation, although a reduced rate of conversion of cholecalciferol to 25(OH)D may also contribute. Differences in levels of plasma 25(OH)D may contribute to the greater incidence of and mortality from colorectal cancer seen among African Americans.[30]

In the United States, colorectal cancer mortality follows a latitudinal gradient, with higher mortality rates seen in individuals who reside at higher latitudes.[31-34] A large observational study in Norway found that people diagnosed with colorectal cancer in the summer and autumn, when 25(OH)D concentrations are highest, had significantly better survival than those diagnosed in the winter.[35,36] We previously showed that higher prediagnosis plasma levels of 25(OH)D and higher postdiagnostic 25(OH)D scores are associated with significant reductions in mortality among patients with established colorectal cancer, although, in those studies, a substantially greater proportion of the population had plasma 25(OH)D levels higher than 33 ng/mL.[15,16]

In this study, we did not detect an association between higher plasma 25(OH)D and patient outcome. It is possible that vitamin D may have limited impact on the natural history of colorectal cancer once it has metastasized. Preclinical data indicate that VDR expression is decreased

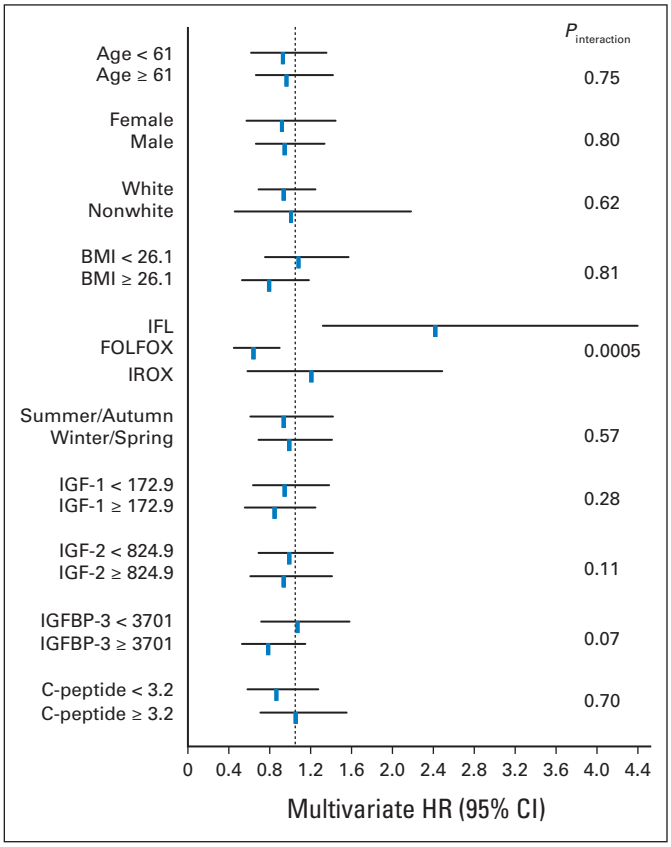

Fig 3. Multivariate hazard ratios (HR) and 95% CI for death (overall survival) across strata of various factors, comparing extreme quartiles of plasma 25-hydroxyvitamin D. BMI, body-mass index in kg/m^2; IFL, irinotecan, bolus fluorouracil (FU), leucovorin (LV); FOLFOX, infusional FU, LV, oxaliplatin; IROX, irinotecan, oxaliplatin; IGF, insulin-like growth factor; IGFBP, IGF binding protein.

in late stages of neoplasia,[37] perhaps leading to loss of response of colon tumor cells to vitamin D. Yet another potential explanation for the discrepant results is that our study had limited statistical power, with only a small number of patients with plasma 25(OH)D levels sufficient for a protective effect on cancer outcome.[24] Indeed, based on the distribution of vitamin D levels ultimately found in this cohort, we had only a 15% to 20% power[38] to detect an improvement in overall survival comparable to the findings of prior studies.[15,16]

Our exploratory analyses revealed that patients with stage IV colorectal cancer randomly assigned to FOLFOX may have improved survival with higher baseline levels of 25(OH)D, whereas those randomly assigned to IFL had worse survival. Given the limited number of patients in this subgroup analysis, our findings may simply be due to chance. However, in vitro and in vivo analyses suggest that 1,25(OH)2D may potentiate the antineoplastic effects of platinum agents.[39-41] We also considered the possibility that vitamin D may have a greater impact on outcome in tumors that respond to chemotherapy, but our results did not change when we adjusted our analysis for patients with confirmed tumor response at 18 weeks. These findings in patients on FOLFOX require confirmation in other cohorts. We also detected a trend toward an interaction between plasma 25(OH)D levels and baseline plasma IGFBP-3. Given that higher levels of IBFBP-3 were previously found to be significantly associated with patient outcome in this cohort,[20] further study of the interaction between the vitamin D and IGF pathways is warranted.

Analyzing patients participating in a National Cancer Institute–sponsored trial, we were able to assess plasma 25(OH)D levels across a large cohort of patients with stage IV colorectal cancer across North America. In addition, the vitamin D assay that we used had high precision. Nonetheless, several potential limitations deserve comment. Baseline 25(OH)D levels may be influenced by morbidity from recent surgery or burden of cancer, particularly in the liver, and may therefore not reflect true vitamin D status. However, as part of the clinical trial, all patients were required to have adequate biochemical parameters for enrollment, including total bilirubin $\leq$ 1.5 times the institutional upper limit of normal and AST and alkaline phosphatase $\leq$ 5 times the institutional upper limit of normal.[17] Moreover, vitamin D levels were not significantly associated with liver function tests or the presence of liver metastases. Consequently, it is unlikely that the low levels of plasma 25(OH)D seen in our cohort are explained by the presence of liver metastases. Another potential limitation is that plasma 25(OH)D levels were only measured at the start of chemotherapy; as such, the impact of changes in these levels on patient outcome could not be assessed.

In conclusion, we found a high prevalence of vitamin D insufficiency ($>$ 80%) among patients with metastatic colorectal cancer undergoing first-line chemotherapy, particularly in female and black patients. Ultimately, the relative inadequacy of vitamin D levels in this population may have precluded a robust examination of the association between baseline levels and patient outcome. Further study of the impact of optimizing vitamin D status in patients with stage IV colorectal cancer is warranted.

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