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25-Hydroxyvitamin D Levels and Survival in Advanced Pancreatic Cancer: Findings From CALGB 80303 (Alliance)

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Background	Data from animal and cell-line models suggest that vitamin D metabolism plays an important role in pancreatic tumor behavior. Although vitamin D deficiency has been implicated in numerous cancers, the vitamin D status of patients with advanced pancreatic cancer and the effect of baseline vitamin D levels on survival are unknown.
Methods	Participants in this correlative study (CALGB 151006) were enrolled in CALGB 80303, which was a randomized trial of patients with advanced pancreatic cancer that demonstrated no difference in overall survival (OS) among patients treated with gemcitabine plus placebo vs gemcitabine plus bevacizumab. We measured baseline serum 25-hydroxyvitamin D (25[OH]D) levels and examined associations between baseline 25(OH)D levels and progression-free survival and OS using the Cox rank score test. All statistical tests were two-sided.
Results	Of 256 patients with available serum, the median 25(OH)D level was 21.7 ng/mL (range 4 to 77). 44.5% of patients were vitamin D deficient (25[OH]D <20 ng/mL), and 32.4% were insufficient (25[OH]D ≥20 and <30 ng/mL). 25(OH)D levels were lower in black patients compared with white patients, and patients of other/undisclosed race (10.7 vs 22.4 vs 20.9 ng/mL, $P < .001$). Baseline 25(OH)D levels were not associated with PFS (HR = 1.00, 95% CI = 0.99 to 1.01, $P = .60$) or OS (HR = 1.00, 95% CI = 0.99 to 1.01, $P = .95$).
Conclusion	Vitamin D deficiency was highly prevalent among patients with a new diagnosis of advanced pancreatic cancer. Black patients had statistically significantly lower 25(OH)D levels than white patients. In this cohort of patients with advanced pancreatic cancer receiving gemcitabine-based chemotherapy, baseline 25(OH)D levels were not associated with PFS or OS.

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The biologically active form of vitamin D, calcitriol (1,25[OH]$_2$D), is a pleiotropic steroid hormone. In addition to its role in calcium homeostasis, it affects proliferation and differentiation of many cell types, including breast, prostate, and colon cancers (1–4). Its effects on pancreatic cancer cells are less well understood, although studies have reported growth arrest in pancreatic cell lines in response to 1,25(OH)$_2$D. In vitro, 1,25(OH)$_2$D analogues inhibit pancreatic cancer cell proliferation, induce differentiation, and promote apoptosis (5–9). In vivo, they inhibit growth of pancreatic tumor xenografts in immunodeficient mice (8,10).

Scientific observations from animal and cell-line models on pancreatic cancer pathogenesis support the hypothesis that vitamin D metabolism is also important in tumor maintenance and may contribute in part to this tumor's chemoresistance. Specifically, the vitamin D receptor (VDR) is known to mediate the activity of vitamin D and has been identified in pancreatic cancer cells (8,11). In one study of VDR expression in human pancreatic cancer cell lines, it was established that VDR expression was increased more than

threefold compared with that in normal pancreatic cells (12). Both normal and malignant pancreatic cells express 1-α-hydroxylase, the enzyme that converts plasma 25(OH)D into its bioactive form 1,25-dihydroxycholecalciferol (1,25[OH]$_2$D) (11,13,14).

Recent data from two genome-wide analysis studies (GWAS) point to the role of the VDR and vitamin D pathway in patients with pancreatic adenocarcinoma. GWAS performed on a cohort of patients enrolled on Cancer and Leukemia Group B (CALGB) 80303 and on patients included in the Pancreatic Cancer Cohort Consortium (PanScan) identified one VDR single-nucleotide polymorphism (SNP) that associates with better overall survival (OS) (15).

To our knowledge, no study has previously investigated whether vitamin D concentration is predictive of survival following a diagnosis with pancreatic cancer. We examined the cohort of participants in CALGB 80303 who had advanced pancreatic cancer and received systemic chemotherapy in the context of this randomized clinical trial. We aimed to describe the prevalence of vitamin D

deficiency at baseline and to examine the association of vitamin D status with outcomes.

Method

Study Population
Participants in this correlative study (CALGB 151006) were enrolled in CALGB 80303, which was a national, multi-center, double-blind phase III study that randomly assigned patients (1:1) with advanced pancreatic cancer to gemcitabine plus bevacizumab vs gemcitabine plus placebo. Full details of the trial and results were previously published ([16]). Eligible patients had unresectable adenocarcinoma of the pancreas and had received no prior therapy for metastatic disease (prior radiation and/or chemotherapy in the adjuvant setting were allowed). A baseline Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2 and adequate renal, liver, and bone marrow function were required.

Patients signed informed consent for participation in the therapeutic trial and were given the option to sign a secondary consent for a companion study for biomarker research. This correlative study was approved by the UCSF Committee for Human Research (IRB Number 10-04423).

In total, 602 patients were enrolled in CALGB 80303, and 535 were treated. Three hundred and fifty-two patients provided optional consent for collection of blood and urine specimens, of whom 274 had serum available in storage. Figure 1 illustrates the derivation of the final cohort size (n = 256) and reasons for exclusion at various time points. Among the 535 patients treated on study, the median OS was 5.8 months in the gemcitabine plus bevacizumab arm and 5.9 months in the gemcitabine plus placebo arm. With respect to the primary outcome, consented patients with available serum samples were representative of the larger study population with similar median OS (5.9 vs 5.8 months, respectively).

Response and Progression Criteria
Study enrollment required documentation of disease extent by CT scan prior to initiation of therapy. Patients were evaluated every eight weeks for response, which was assessed through conventional RECIST criteria. Progression-free survival (PFS) was calculated as time from study enrollment to disease progression or death. OS was calculated as the time from registration to death from any cause.

Serum Biomarker Assessment
Blood samples were collected from consenting participants in CALGB 80303 at the time of study registration at respective institutions and shipped to the CALGB Pathology Coordinating Office for storage (Columbus, OH). For processing of the 25(OH)D assay, 150 microliters of available serum specimens were placed into Sarstedt #72.730 cryovials and shipped on dry ice to Heartland Assays, Inc. (Ames, IA). It has been previously established that 25(OH)D results obtained for patients are unaffected by multiple freeze-thaw cycles ([17]).

The method for quantitative determination of 25(OH)D was a Food and Drug Administration-approved direct, competitive chemiluminescence immunoassay using the DiaSorin© LIAISON 25-OH Vitamin D Total assay ([18],[19]). This system was selected
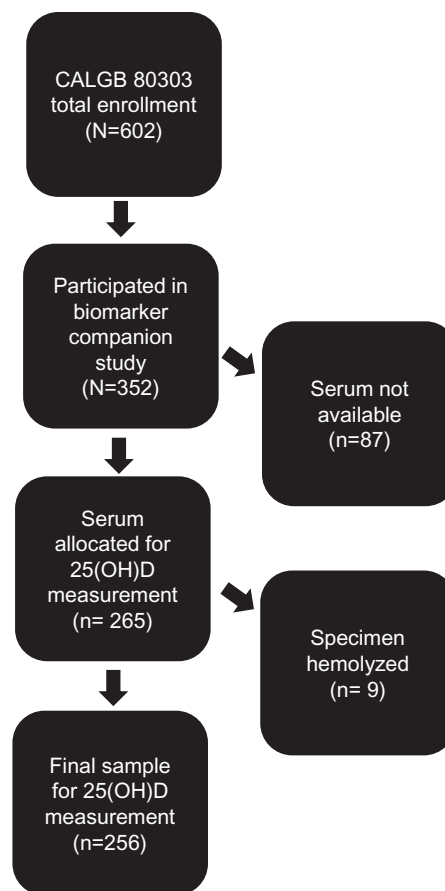

Figure 1. Derivation of cohort size.

based upon its high sample throughput and equal recognition of circulating $25(OH)D_2$ and $25(OH)D_3$. All values in our data set were above the assay's minimum detectable concentration of less than 2.5 ng/mL ([18]).

To ensure quality control, 5% of samples were submitted as blinded duplicates to verify reproducibility of assay results (n = 13). All laboratory personnel were blinded to patient identifiers and outcome. The Bland-Altman plot of 13 duplicate specimens is displayed in Supplementary Figure 1 (available online). The intra-class correlation coefficient of the assay was calculated to be 0.97 (95% CI = 0.91 to 0.99).

Statistical Design and Analysis
Descriptive analyses were performed to report the demographic and clinical characteristics. Although there is no consensus regarding optimal physiologic levels, we defined vitamin D levels as they are most commonly reported in the literature: vitamin D deficiency was defined as a serum 25(OH)D level less than 20 ng/mL, insufficiency as 20 to 29 ng/mL, and sufficiency as 30 ng/mL and greater ([20]). The association between vitamin D levels and patient characteristics was described with medians and ranges and compared using the Wilcoxon rank sum test ([21]). The cut point for age (<70 and ≥70 years) is an optimized value determined by a recursive partitioning approach ([22]). The cut point for body-mass index (BMI) (<25 and ≥25 kg/m²) was determined by the median value. A strong concordance between genetically estimated ancestry and

self-reported race was previously reported in the GWAS of this cohort (23). To address the possibility of reporting bias for the self-reported variable of race, analyses were also performed on the subset of patients with available GWAS data on genetically estimated race.

The primary statistical objective was to investigate the association between 25(OH)D level and OS. The association was tested using the Cox rank score test, using 25(OH)D as a continuous variable (eg, per each incremental increase of 1 ng/mL) (24). Serum 25(OH)D levels were defined in four quartiles, with almost equal numbers of patients in each. Chi-square tests were used to compare the impact of demographic and clinical characteristics across quartiles (25). The distributions of OS and PFS were also compared between the subgroups of patients with extreme low 25(OH)D levels (<10 ng/mL) and "optimal" levels (≥36 ng/mL) (26) using a log rank test (27).

In secondary analyses, tests of interaction between serum 25(OH)D and potential effect modifiers were assessed by entering in the model 25(OH)D as a continuous variable with covariates as either continuous or binary variables. Geographic latitude as determined by home zipcode was tested both as a binary variable and as

a continuous variable; the Kendall's rank correlation test was used to test latitude as a continuous variable (21). Additional exploratory analyses using the Cox proportional hazards model and ensemble methods (eg, random forests) were employed (22,28). Differences in 25(OH)D levels among racial populations were tested using the Kruskal-Wallis test (29). The reported P values and confidence intervals are two sided and not adjusted for multiple testing.

All analyses were performed using R software (version 3.0.1) and extension packages for survival (version 2.37–4), party (version 1.0–8), and zipcode (version 1.0) (22,30–32). The intraclass correlation coefficient of the 25(OH)D assay was calculated using the ICC function of the irr extension package (33). Data collection and statistical analyses were conducted by the Alliance Statistics and Data Center. All analyses were based on the study database frozen on June 9, 2009.

Results

Patient Characteristics

Baseline characteristics of the 256 included patients are displayed in Table 1. The median age at diagnosis was 64 years (range 35 to

Table 1. Relationship of 25(OH)D levels to selected patient characteristics

Characteristic	No. patients (%)	Median 25(OH)D, ng/mL (range)	P
Age at diagnosis, y*			
<70	182 (71.1)	21.4 (4.0–50.2)	>.15
≥70	74 (28.9)	22.4 (5.3–77.0)	
Sex			
Female	120 (46.9)	20.8 (4–47)	>.07
Male	136 (53.1)	22.5 (4–77)	
Race			
White	225 (87.9)	22.4 (4–77)	<.001
Black	23 (9.0)	10.7 (4.0–36.3)	
Other/Unknown	8 (3.1)	20.9 (12.6–31.8)	
BMI, kg/m²†			
<25	126 (49.2)	21.6 (4.0–71.2)	>.47
≥25	130 (50.8)	22.3 (4.2–77)	
ECOG PS			
0			
1	234 (91.4)	22.1 (4–77)	>.18
2	22 (8.6)	18.8 (4–42.4)	
Extent of disease			
Locally advanced	217 (84.8)	21.3 (4–77)	>.17
Metastatic	39 (15.2)	22.6 (9.5–71.2)	
Prior radiation			
No	225 (87.9)	22 (4–77)	>.12
Yes	31 (12.1)	17.1 (7.5–40.8)	
Prior chemotherapy			
No	223 (87.1)	22 (4–77)	>.16‡
Yes	28 (10.9)	16.55 (7.5–40.8)	
Unknown	5 (2.0)	24.9 (5.9–33.1)	
Geographic latitude			
<40°	119 (46.5)	21.9 (4–71.2)	>.89‡
≥40°	96 (37.5)	20.7 (4.2–43.3)	
Unknown	41 (16)	21.3 (9.5–77)	

* Cut point is an optimized values decided by using recursive partitioning approach. 25(OH)D = 25-hydroxyvitamin D₃; BMI=Body-mass index; ECOG PS = Eastern Cooperative Oncology Group Performance Status.

† Body-mass index is calculated as weight (kilograms) divided by height (meters) squared. Values reflect patient status at time of initiation of chemotherapy (clinical trial enrollment). Cut point is the median value.

‡ Comparison did not include the Unknown group.

84). 29% of participants were 70 years or older, 53% were men, and 88% were self reported as white. GWAS data on genetic race was available for 211 of 256 participants; of these, genetic race was reported as white for 187 (88%), black for 14 (6.6%), and unknown for 10 (4.7%).

Distribution of 25(OH)D Levels

The median serum 25(OH)D level for the entire cohort was 21.7 ng/mL (range 4 to 77). At time of specimen collection prior to initiation of chemotherapy, 44.5% of patients were vitamin D deficient (25[OH]D <20 ng/mL), and 32.4% were insufficient (25[OH]D ≥20 and <30 ng/mL). Only 23% of patients had serum 25(OH)D levels above the threshold for vitamin D sufficiency (≥30 ng/mL). Figure 2 summarizes the distribution of serum 25(OH)D levels.
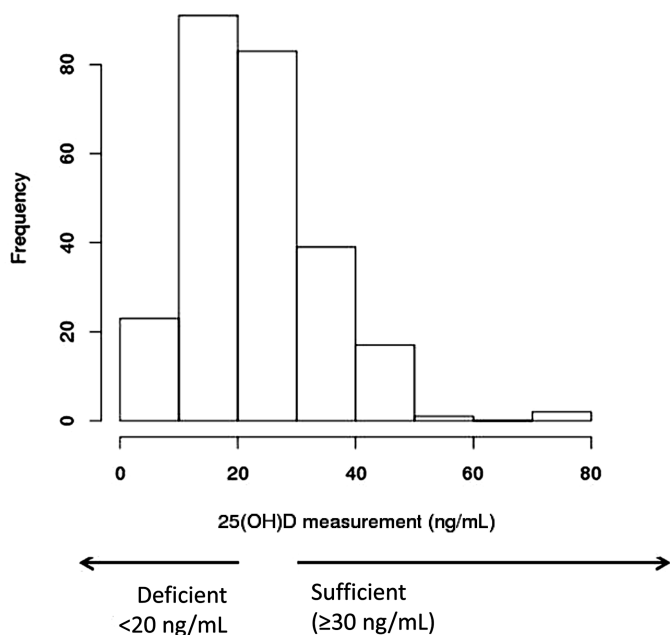

Figure 2. Distribution of serum 25(OH)D levels in the study cohort (n = 256).

Association of 25(OH)D Levels With Patient Characteristics

Among 256 patients with available serum, 25(OH)D levels were lower in patients self-reported as black (median 10.7 ng/mL) compared with white patients (median 22.4 ng/mL) and patients of other or undisclosed race (median 20.9 ng/mL) (P < .001). Vitamin D deficiency was more common among self-reported black patients (82.6%) compared with white patients (40.9%) and those of other or undisclosed race (37.5%). Moreover, only 4.3% of blacks, 25.3% of whites, and 12.5% of patients of other or undisclosed race demonstrated serum 25(OH)D levels in a sufficient range. Boxplots of 25(OH)D measurements according to race are shown in Figure 3. Among the 211 patients for whom both serum and GWAS data on race were available, 25(OH)D levels according to genetic race were comparable to those for self-reported race. 25(OH)D levels were statistically significantly lower in genetically black patients (median 10.1 ng/mL) than in genetically white patients (median 22.4 ng/mL) (P < .001).

As shown in Table 1, baseline serum 25(OH)D concentration was not statistically significantly associated with patient age, sex, BMI, ECOG performance status, extent of disease, prior radiation, prior chemotherapy, or geographic latitude (as a binary variable). When tested as a continuous variable, geographic latitude was still not associated with 25(OH)D levels (P = .95).

In a secondary analysis, patients were grouped into four quartiles of 63 to 65 patients apiece, according to escalating serum 25(OH)D levels (Quartile 1: 25(OH)D <15.3; Quartile 2: 25(OH)D ≥15.3 and <21.7 ng/mL; Quartile 3: 25(OH)D ≥21.7 and <29 ng/mL; Quartile 4: 25(OH)D ≥29 ng/mL). Groupings were not entirely equal because of ties in 25(OH)D levels at cut points. We further examined the association of four quartiles of 25(OH)D levels with potential covariables (Table 2). Again, there was no interaction with any covariable except race. This finding was reproduced using GWAS data on genetic race for 211 patients (P < .001). Of the 23 self-reported black patients included in this study, 16 (70%) were in the lowest quartile of serum 25(OH)D levels and only one (4%) was in the highest quartile. By comparison, the
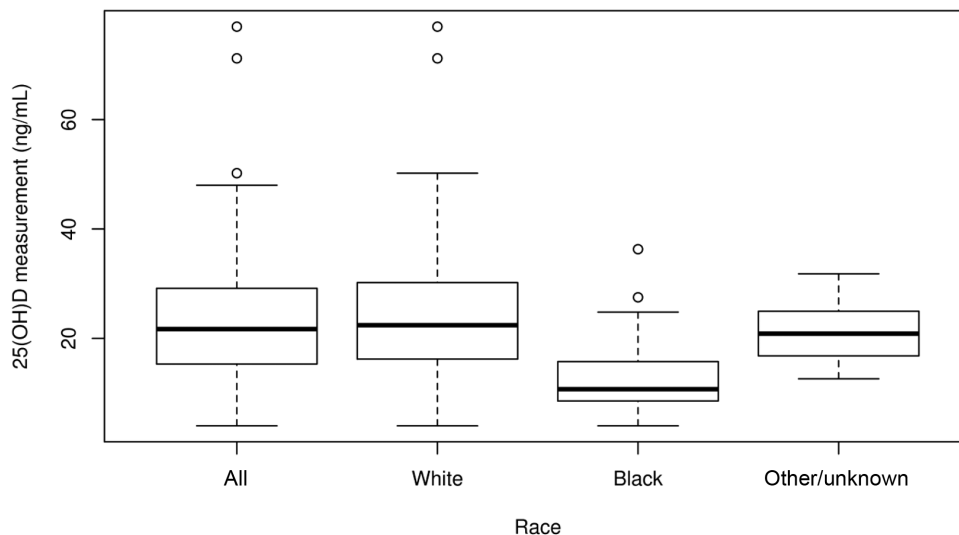

Figure 3. Boxplots of 25(OH)D measurements, according to race. **Solid horizontal lines** represent medians and **whiskers** represent the ranges.

Table 2. Characteristics of study cohort according to quartile of serum 25(OH)D (n = 256)*

	Serum 25(OH)D				
	Quartile 1	**Quartile 2**	**Quartile 3**	**Quartile 4**	
Characteristic	**N = 63**	**N = 64**	**N = 64**	**N = 65**	***P***
Serum 25(OH)D, ng/mL					
Mean	10.6	18.0	24.8	37.8	<.001
Age at diagnosis, y					
Mean	61.8	65.4	62.9	64.6	.19
BMI, kg/m²					
Mean	26	26	28	26	.98
Sex, No.					
Female	37	26	30	27	
Male	26	38	34	38	.15
Race or ethnicity, No.					
White	46	56	60	63	
Black	16	4	2	1	
Other/unknown	1	4	2	1	<.001
ECOG PS, No.					
0					
1	56	56	62	60	
2	7	8	2	5	.23
Extent of disease, No.					
Metastatic	56	55	54	52	
Locally advanced	7	9	10	13	.56
Prior radiation, No.					
No	55	54	56	60	
Yes	8	10	8	5	.58
Prior chemotherapy, No.					
No	52	56	55	60	
Yes	9	8	7	4	.48†
Unknown	2	0	2	1	
Geographic latitude, No.					
<40°	34	23	32	30	
≥40°	20	29	23	24	.26†
Unknown	9	12	9	11	

* Body mass index is calculated as weight (kilograms) divided by height (meters) squared. Values reflect patient status at time of initiation of chemotherapy (clinical trial enrollment). Quartile 1: 25(OH)D<15.3 ng/mL; Quartile 2: 25(OH)D ≥15.3 and <21.7 ng/mL; Quartile 3: 25(OH)D ≥21.7 and <29 ng/mL; Quartile 4: 25(OH)D ≥29 ng/mL. 25(OH)D = 25-hydroxyvitamin D_3; BMI = body mass index; ECOG PS = Eastern Cooperative Oncology Group Performance Status.

† Comparison did not include the Unknown group.

white patients included in this study were uniformly represented in each quartile.

### Relationship of Vitamin D Levels on Patient Outcomes

While only 23% of our cohort had sufficient 25(OH)D levels (≥30 ng/mL) at study baseline, we still examined the association between baseline levels and patient outcomes, including PFS and OS. At the time of this analysis, 254 of 256 patients were deceased, with an event rate of 97%. The unadjusted HR for PFS was 1.00 (95% CI = 0.99 to 1.01, *P* = .60). The unadjusted HR for OS was 1.00 (95% CI = 0.99 to 1.01, *P* = .95). After adjustment for race, baseline 25(OH)D levels were not associated with PFS (HR = 1.00, 95% CI = 0.99 to 1.01, *P* = .76) or OS (HR = 1.00, 95% CI = 0.99 to 1.01, *P* = .97). No statistically significant relationships were detected between quartiles of serum 25(OH)D levels and either PFS or OS. Kaplan-Meier curves for PFS and OS, according to quartile, are displayed in Figure 4.

Patients with serum 25(OH)D levels in the highest quartile had a PFS of 3.88 months (95% CI = 3.6 to 5.5 months) and OS of 5.36 months (95% CI = 4.7 to 8.6 months). Patients with serum 25(OH)D levels in the lowest quartile had a PFS of 2.56 months (95% CI = 2.0 to 4.0) and OS of 5.95 months (95% CI = 4.6 to 9.1). Based upon the observation of increasing median PFS across quartiles (2.56, 2.78, 3.60, and 3.88 months in Quartiles 1, 2, 3, and 4, respectively), we performed log rank score tests for trend. A long rank score test of 25(OH)D level as a continuous variable did not demonstrate an association with PFS (*P* = .60). No association for PFS was seen by testing 25(OH)D levels according to rank or quartiles (*P* = .38 and *P* = .19, respectively).

Among the 34 patients with levels of 25(OH)D defined as physiologically "optimal" (≥36 ng/mL) (26), the median PFS was 3.84 months (95% CI = 2.0 to 4.9) and median OS was 4.99 months (95% CI = 3.98 to 8.05). Among patients with extreme low levels (<10 ng/mL), the median PFS was 1.99 (95% CI = 1.61 to 4.57) and median OS was 4.80 months (95% CI = 3.42 to 9.13). When the subgroups with extreme high or low levels were compared, no statistically significant differences in PFS (*P* = .48) or OS (*P* = .79) were seen.
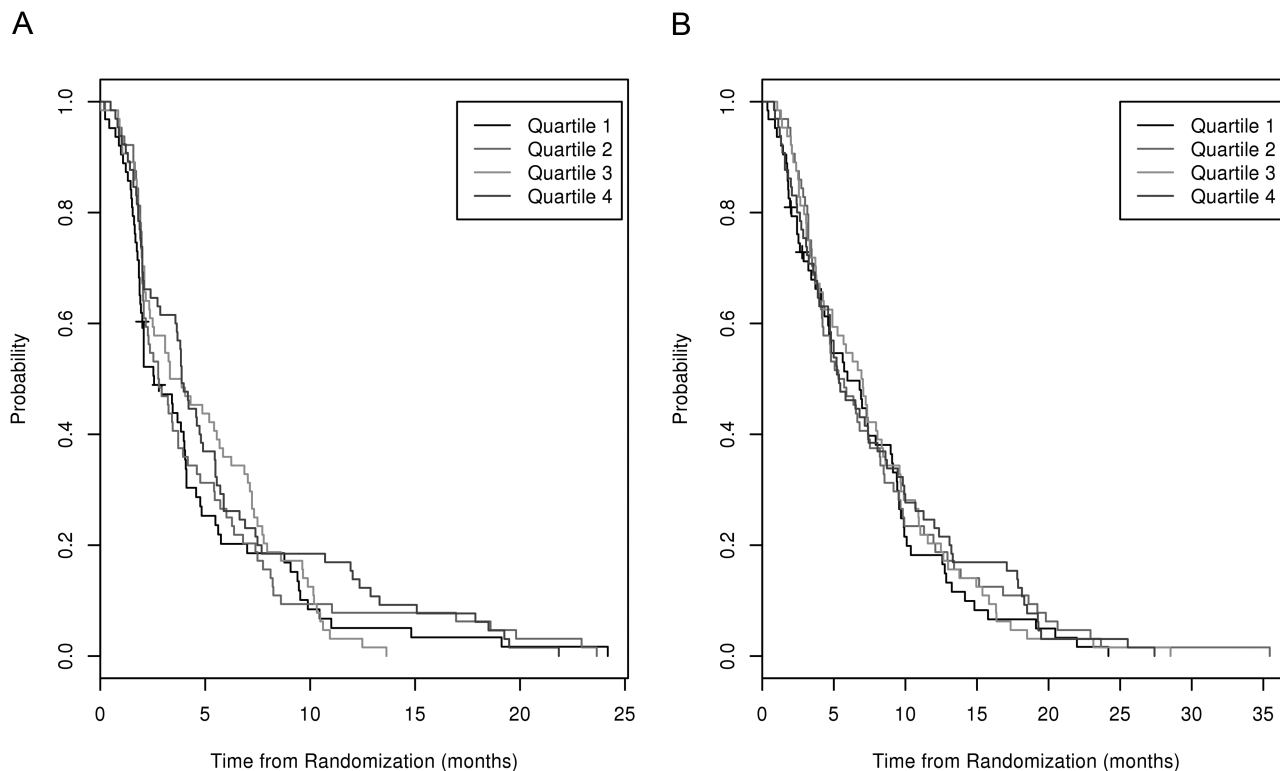
Figure 4. **A)** Progression-free survival (PFS) by quartile of 25(OH)D. **B)** Overall survival by quartile of 25(OH)D.

Discussion

This study is the first to characterize the vitamin D status of patients at time of diagnosis with advanced pancreatic cancer and to test the association between 25(OH)D levels and OS. In the cohort of patients we studied, 44.5% were vitamin D deficient (<20 ng/mL) and 22.5% were vitamin D insufficient (<30 ng/mL). Among patients self-reported as black, serum 25(OH)D levels were statistically significantly lower than in patients self-reported as white. However, low vitamin D levels were not associated with diminished OS or PFS.

The high prevalence of vitamin D insufficiency in our cohort is consistent with reports from cohorts of patients with breast cancer and metastatic colorectal cancer. A recent study of women with stages I-III breast cancer, which included a high proportion of black and Hispanic women, reported that 74% of women were deficient at baseline and also found that supplementation using standard dosing guidelines was inadequate to increase 25(OH)D levels to a normal range (34). In a cohort of 515 patients with metastatic colorectal cancer, 50% were vitamin D deficient, and an additional 32% were insufficient (35). In both of these cohorts, 25(OH)D levels were lower in black patients compared with white patients and patients of other race (34,35).

Low vitamin D levels are not unique to cancer patients, however. Among 14 000 individuals who participated in the National Health and Nutrition Examination Survey (NHANES) III between 2001 and 2004, 77% had 25(OH)D levels less than 30 ng/mL, with a mean level of 24 ng/mL (36). In comparison with the NHANES population, we detected a higher prevalence of extreme low serum 25(OH)D levels (<10 ng/mL) in our study cohort (9.1% vs 6%).

The apparent propensity toward extreme deficiency in patients with advanced pancreatic cancer may reflect malabsorption of this fat-soluble vitamin in those with pancreatic exocrine insufficiency. However, vitamin D concentration is primarily dependent on endogenous production by skin following exposure to ultraviolet rays and, to a lesser extent, on dietary consumption of vitamin D (37,38). The greater prevalence of vitamin D deficiency among black patients in our cohort is consistent with other studies (34–36,39) and likely due to reduced vitamin D photosynthesis in pigmented skin because of greater melanin content and increased absorption of UVB radiation (40). Between 2006 and 2010, black Americans had an age-adjusted incidence rate of pancreatic cancer that was 31% higher than in white Americans and an age-adjusted mortality rate that was 27% higher (41). In the NHANES III cohort, non-Hispanic black race was identified as an independent predictor of pancreatic cancer-related mortality (adjusted HR = 2.80, 95% CI = 1.23 to 6.37) (42). A recent review on racial disparities for a variety of cancer types identified pancreatic cancer as one of several cancers where increased risk among blacks was not attributable to socioeconomic status, cancer stage at diagnosis, or treatment (43). Our data do not suggest that racial disparities in survival for patients with advanced pancreatic cancer can be explained by 25(OH)D levels alone.

The published data on the prognostic role of circulating 25(OH)D levels in other cancers are generally sparse and of poor quality. In a systematic review of all published studies evaluating the impact of baseline 25(OH)D on outcome measures in a multitude of diagnoses and disease stages, five of eight demonstrated a statistically significant negative prognostic role for low 25(OH)D levels, and three demonstrated no prognostic role (44). Two studies on early-stage breast cancer produced conflicting results, and two studies

on non-small cell lung cancer reported no prognostic role. Studies that demonstrated a prognostic effect of 25(OH)D levels on outcome measures included a study in melanoma and a smaller study in prostate cancer. Two studies in colon cancer reported a prognostic role for 25(OH)D; however, a larger study of 515 clinical trial participants was published following this review that showed no relationship between 25(OH)D levels and patient outcomes (35).

In the current study, we did not detect an association between serum 25(OH)D levels and patient outcome. It is possible that vitamin D may have a limited impact on the natural history of pancreatic cancer given the grim prognosis associated with advanced stage disease. On the other hand, our data may underestimate the actual prevalence of vitamin D insufficiency associated with advanced pancreatic cancer, because CALGB 80303 participants met rigorous eligibility criteria (eg, 90% of subjects had an ECOG performance status of 0 or 1). This suggests that most patients with advanced pancreatic cancer may be vitamin D insufficient, or worse. As opposed to studies emanating from single institutions, this analysis of patients participating in a National Cancer Institute-sponsored trial allowed for assessment of serum 25(OH)D levels in patients living at various latitudes across North America. In addition, the vitamin D assay that was utilized had high precision.

Nonetheless, potential limitations should be addressed. This analysis included a small number of patients who were categorized as having sufficient 25(OH)D levels, which may have prevented a robust examination of association with outcomes. Baseline 25(OH)D levels in cancer patients may not represent true steady state but rather could reflect inadequate nutrition or limited outdoor activity as influenced by morbidity from recent illness and/or burden of cancer (45). Because this study only performed 25(OH)D measurement on specimens obtained prior to the initiation of gemcitabine-based chemotherapy, the impact of chemotherapy on vitamin D status and its possible effect on patient outcomes could not be studied.

In conclusion, we detected a high prevalence of vitamin D insufficiency among patients with advanced pancreatic cancer undergoing gemcitabine-based chemotherapy. We found that vitamin D deficiency was pronounced among black patients and substantiated this finding using GWAS data for race. This observation suggests the possibility of a genetic basis for the variability between 25(OH)D levels and exposure. While the relative rarity of adequate vitamin D levels in this population may preclude detection of an association between higher vitamin D levels and patient outcomes, additional inquiry regarding genetic factors that may predict for benefit from optimized vitamin D status in patients with pancreatic cancer may be considered.

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