Published OnlineFirst July 17, 2018; DOI: 10.1158/1078-0432.CCR-18-0483

Clinical Cancer Research

Vitamin D Supplementation and Survival of Patients with Non–small Cell Lung Cancer: A Randomized, Double-Blind, Placebo-Controlled Trial

Tadashi Akiba[1], Toshiaki Morikawa[1], Makoto Odaka[1], Takeo Nakada[1], Noriki Kamiya[2], Makoto Yamashita[1], Mitsuo Yabe[1], Takuya Inagaki[1], Hisatoshi Asano[1], Shohei Mori[1], Yo Tsukamoto[1], and Mitsuyoshi Urashima[3]

Abstract

Purpose: Higher serum 25-hydroxyvitamin D (25(OH)D) levels are reportedly associated with better survival in early-stage non–small cell lung cancer (NSCLC). Therefore, whether vitamin D supplementation can improve the prognosis of patients with NSCLC was examined (UMIN000001869).

Experimental Design: A randomized, double-blind trial comparing vitamin D supplements (1,200 IU/day) with placebo for 1 year after operation was conducted. The primary and secondary outcomes were relapse-free survival (RFS) and overall survival (OS), respectively. Prespecified subgroup analyses were performed with stratification by stage (early vs. advanced), pathology (adenocarcinoma vs. others), and 25(OH)D levels (low, <20 ng/mL vs. high, ≥20 ng/mL). Polymorphisms of vitamin D receptor (VDR) and vitamin D–binding protein (DBP) and survival were also examined.

Results: Patients with NSCLC ($n = 155$) were randomly assigned to receive vitamin D ($n = 77$) or placebo ($n = 78$) and followed for a median of 3.3 years. Relapse and death occurred in 40 (28%) and 24 (17%) patients, respectively. In the total study population, no significant difference in either RFS or OS was seen with vitamin D compared with the placebo group. However, by restricting the analysis to the subgroup with early-stage adenocarcinoma with low 25(OH)D, the vitamin D group showed significantly better 5-year RFS (86% vs. 50%, $P = 0.04$) and OS (91% vs. 48%, $P = 0.02$) than the placebo group. Among the examined polymorphisms, DBP1 (rs7041) TT and CDX2 (rs11568820) AA/AG genotypes were markers of better prognosis, even with multivariate adjustment.

Conclusion: In patients with NSCLC, vitamin D supplementation may improve survival of patients with early-stage lung adenocarcinoma with lower 25(OH)D levels. *Clin Cancer Res; 1–9.* ©2018 AACR.

Introduction

Age-standardized 5-year survival of patients with lung cancer is typically low, in the range of 10% to 30%, in most geographical areas (1). Recently, molecular targeting immunotherapies have been shown to prolong survival in advanced non–small cell lung cancer (NSCLC; refs. 2–5). However, in early-stage NSCLC, there is no curative treatment except for surgical resection. Despite adding adjuvant chemotherapy to surgery, relapse rates are still high. Thus, a novel treatment to prevent relapse that is less toxic and cost-effective, such as the use of natural compounds like vitamin D, should be developed for early-stage NSCLC.

Vitamin D obtained from skin exposed to sunlight, diet, or a supplement is metabolized in the liver to 25-hydroxyvitamin D (25(OH)D), which is used as a biomarker of vitamin D status. 25(OH)D is further activated in the kidneys by 1α-hydroxylase to 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), which facilitates calcium absorption and is associated with bone health. In addition, most tissues, as well as cancers, have both 1α-hydroxylase to convert serum 25(OH)D to $1,25(OH)_2D$ and vitamin D receptor (VDR), a steroid hormone nuclear receptor that regulates a variety of genes within a cell (6), by which vitamin D is hypothesized to prevent cancer relapse through inhibiting cell proliferation, angiogenesis, and metastasis, while inducing apoptosis and differentiation (7).

Indeed, patients with early-stage NSCLC who had surgery during the summer with the highest vitamin D intake, or with a higher serum 25(OH)D, had a better prognosis than patients who had surgery during the winter with the lowest vitamin D intake, or with lower 25(OH)D (8, 9). On the other hand, serum 25(OH)D had no association with survival in advanced NSCLC, but VDR SNPs were shown to be significantly associated with survival (10). In addition, SNPs of vitamin D–binding protein (DBP), rs7041 (Asp416Glu; DBP1) and rs4588 (Thr420Lys; DBP2), were found to produce variant

[1]Department of Thoracic Surgery, The Jikei University School of Medicine, Tokyo, Japan. [2]Department of Thoracic Surgery, Kitasato University Kitasato Institute Hospital, Tokyo, Japan. [3]Division of Molecular Epidemiology, the Jikei University School of Medicine, Tokyo, Japan.

Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

This trial was registered as UMIN000001869.

Corresponding Author: Mitsuyoshi Urashima, Jikei University School of Medicine, 3-25-8, Nishi-Shimbashi Minato-ku, Tokyo 105-8461, Japan. Phone: 813-3433-1111; Fax: 815-400-1250; E-mail: urashima@jikei.ac.jp

doi: 10.1158/1078-0432.CCR-18-0483

Published OnlineFirst July 17, 2018; DOI: 10.1158/1078-0432.CCR-18-0483

Translational Relevance

Prospective cohort studies have reported that higher serum 25-hydroxyvitamin D (25(OH)D) levels are associated with better survival in early-stage non–small cell lung cancer (NSCLC). However, because of the studies' observational nature, whether lower 25(OH)D is merely a precursor to relapse and death or a lower 25(OH)D is causally related to shorter survival cannot be determined. To clarify this, a randomized, double-blind, placebo-controlled trial using vitamin D was performed in patients with NSCLC; this is the first trial designed to evaluate the effect of vitamin D on survival of patients with NSCLC. Although vitamin D supplementation was not associated with a difference in survival in the total study population, by restricting the analysis to the subgroup with early-stage adenocarcinoma with 25(OH)D concentrations <20 ng/mL, the vitamin D group showed significantly better 5-year survival than the placebo group. Moreover, polymorphisms of vitamin D–binding protein were also found to influence the survival.

proteins that differ in their affinity for 25(OH)D and $1,25(OH)_2D$ (11–14).

However, because of the studies' observational nature, whether lower 25(OH)D is merely a precursor to relapse and death or a lower 25(OH)D is causally related to shorter survival cannot be determined. To clarify this, a randomized, double-blind, placebo-controlled trial was planned to determine whether vitamin D supplementation could improve relapse-free survival (RFS) and/or overall survival (OS) in the total study population or in prespecified subgroups stratified by stage (early vs. advanced), pathology (adenocarcinoma vs. others), and serum 25(OH)D levels (<20 ng/mL vs. ≥20 ng/mL) before starting supplementation. Furthermore, the effects of VDR and DBP SNPs on RFS and OS were also evaluated.

Patients and Methods

Eligibility

Inclusion criteria were patients: (i) histopathologically diagnosed as having NSCLC (adenocarcinoma, squamous cell carcinoma, adenosquamous carcinoma, or large cell lung carcinoma); (ii) with stage IA to IIIA; (iii) aged 20 to 75 years at entry; (iv) diagnosed and operated at any one of four Jikei University Hospitals at Shimbashi, Kashiwa, Chofu, and Katsushika; (v) with tumor totally resected; (vi) discharged without major complications; and (vii) could visit the Jikei University Hospitals (Tokyo, Japan) and be followed-up for as long as possible. Exclusion criteria were patients: (i) who were already taking a vitamin D supplement or active vitamin D; (ii) who had a history of urinary tract stones; and (iii) who had other difficulties as judged by the surgeon in charge.

Enrollment was done by the collaborating surgeons perioperatively. When patients were considered to satisfy the inclusion criteria, the collaborating surgeons explained the trial to the patients and their families at the hospital outpatient clinic and asked them to participate in the trial. Written, informed consent was obtained from each participant.

Trial design

This was a balanced randomized (1:1), double-blind, placebo-controlled, parallel group study conducted at the Jikei University Hospitals. Participants were enrolled from August 2009 and followed-up. The trial protocol was developed by all authors and approved by the ethics committee of the Jikei University School of Medicine and the clinical study committee of each Jikei hospital (20-231 5521). The trial (UMIN000001869) was registered with the UMIN Clinical Trials Registry on April 2009. The data monitoring center was at the Division of Epidemiology, the Jikei University School of Medicine. Both vitamin D3 and placebo were purchased from Zenyaku Pharmaceutical Co., Ltd. None of the authors had a conflict of interest with the pharmaceutical company making the vitamin D supplements.

In our previous trial in patients with Parkinson disease, a dose of 1,200 IU/day for 1 year raised 25(OH)D levels from 22.5 to 41.7 ng/mL (15), which was considered a relatively higher level of 25(OH)D. Thus, the same dose of 1,200 IU for vitamin D was used in this trial.

Randomization, blinding, intervention, and follow-up

A central computerized procedure generated by an investigator (M. Urashima) who had no clinical involvement in this trial was used to randomly assign patients to permutated blocks of four to receive either vitamin D3 (total 1,200 IU/day) or placebo. The vitamin D and placebo were in capsule form and identical in appearance and taste, containing sesame oil, gelatin derived from swine, and glycerin for placebo or the active supplement. These supplements were prepacked in bottles and consecutively numbered for each patient according to the randomization schedule. The blinding process was performed by office secretaries who monitored and fixed the data, had no clinical involvement in this trial, and did not perform statistical analyses. After obtaining written, informed consent, the patients were assigned to groups, given the corresponding bottles, and asked to take two capsules per day for 12 months at discharge or at an outpatient clinic after operation.

On an outpatient basis, patients were periodically (1–3 months) examined to exclude relapse of lung cancer by interviews, physical examination, chest X-ray, CT, and other examinations as needed. Patients were asked about compliance at every visit. Oral or injection chemotherapy was administered to the patients according to the stage, except in stage IA and tumor size less than 2 cm.

Patients were not permitted to take personal vitamin D supplements. On the other hand, when vitamin D supplementation was medically needed, as for bone fracture or osteoporosis, the supplementation of the trial was stopped.

Vitamin D measurements and genotyping

Serum levels of 25(OH)D were measured by radioimmunoassay at SRL Inc., as described previously (16), before taking the supplement (defined as "Pre") and 1 year after starting the supplement (defined as "Post").

DNA was extracted from peripheral blood samples. DNA fragments were amplified by PCR. The following SNPs of VDR [Fok*I* (rs10735810), Bsm*I* (rs1544410), CDX2 (rs11568820), Apa*I* (rs7976091), and Taq*I* (rs731236)] and vitamin D–binding protein [DBP; DBP1 (rs7041) and DBP2 (rs4588)] were determined by direct sequencing, for which the detailed methods were reported in our previous article (17).

Published OnlineFirst July 17, 2018; DOI: 10.1158/1078-0432.CCR-18-0483

Outcomes

The primary and secondary outcomes were RFS and OS, respectively. RFS was defined as the time from the supplement start date to the earlier date of relapse of cancer or death from any cause. OS was defined as the time from the supplement start date to the date of death from any cause.

Statistical analysis

We hypothesized that 5-year RFS would be 66.66% in the vitamin D group and 50% in the placebo group, with a type I error (two-sided) of 5% and a power of 80%, on the assumption of 10% loss to follow-up. Therefore, 1:1 divided samples of 300 patients with NSCLC were calculated as being sufficient to detect a significant difference. However, because there had been no previous RCTs using vitamin D supplements to improve survival of patients with NSCLC, an interim analysis was planned to reestimate the sample size when the enrolled patient number reached half of the planned sample size. The *P* value for significance at the interim analysis was set as <0.029 according to the Pocock stopping boundaries (18).

To evaluate significant differences between the vitamin D group and the placebo group, Student *t* test was used to analyze continuous variables with normal distributions, and the χ^2 test was used to assess categorical variables of patients' characteristics. Changes in 25(OH)D levels were evaluated by paired *t* test. Kaplan–Meier survival curves were drawn and compared using the log-rank test in an intention-to-treat analysis. Cox proportional hazards models were used to determine HRs and 95% confidence intervals (CIs). Prespecified subgroup analyses were performed with stratification by: stage, early (IA to IIB) versus advanced (IIIA); pathology, adenocarcinoma versus nonadenocarcinoma; and 25(OH)D levels, lower (<20 ng/mL) versus higher (≥20 ng/mL), because most experts consider <20 ng/mL to indicate vitamin D insufficiency (6). Moreover, the effects of five VDRs and two DBP SNPs on survival were evaluated by multivariate analysis with stage, adenocarcinoma, low 25(OH)D, and supplementation with vitamin D. All reported *P* values are two-sided. *P* values <0.05 were considered statistically significant. Stata 14.0 (StataCorp LP) was used for all analyses.

Results

Study population

A flow diagram is shown (Fig. 1). At the midterm analysis in November 2014, 5-year RFS of the vitamin D and placebo groups was much closer than expected. As a result, the reestimated sample size was exploded from 300 to much more than 1,000. Thus, it was decided to stop new enrollment and terminate this trial after further 3-year follow-up. Consequently, a total of 155 patients with NSCLC were randomly assigned to receive vitamin D supplements ($n = 77$) or placebo ($n = 78$) in a double-blind setting for 12 months from August 2009 to November 2014. Because five patients in the vitamin D group and six patients in the placebo group were lost to follow-up, 72 patients in the vitamin D group and 72 patients in the placebo group were analyzed.

In the vitamin D group, the mean levels of 25(OH)D increased significantly from 21 to 39 ng/mL ($P = 0.0001$). On the other hand, the levels did not change significantly, from 22 to 24 ng/mL,

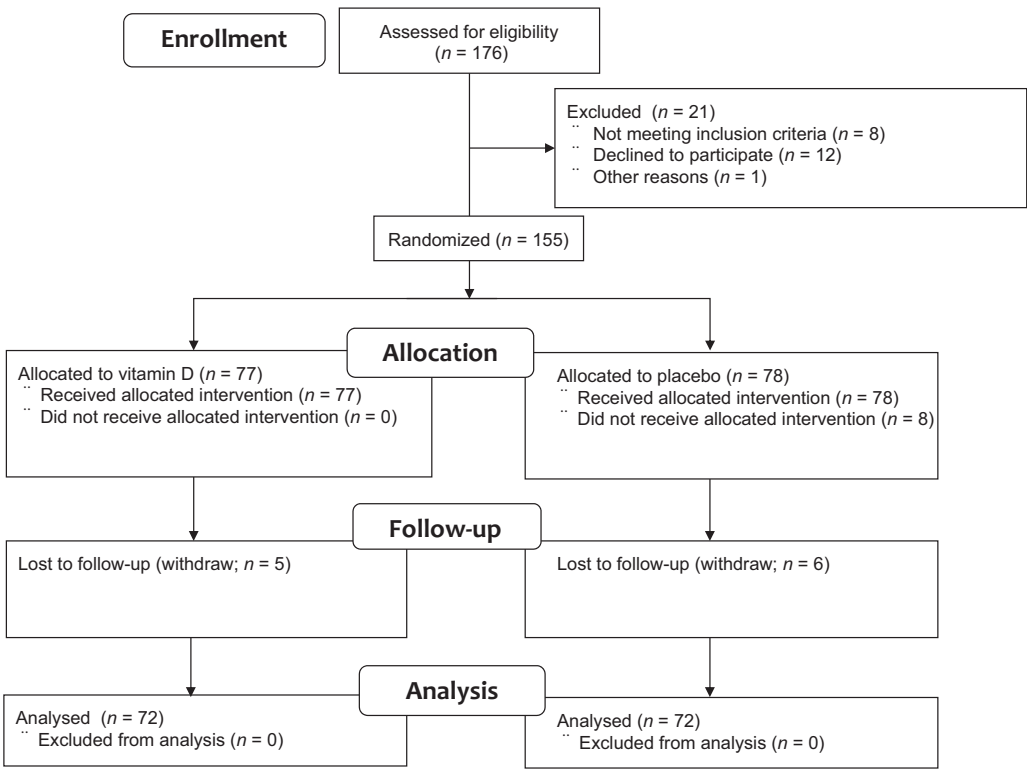

Figure 1.
Flow diagram of vitamin D versus placebo.

Published OnlineFirst July 17, 2018; DOI: 10.1158/1078-0432.CCR-18-0483

Table 1. Patients' characteristics

	Total ($n = 155$)	Vitamin D ($n = 77$)	Placebo ($n = 78$)	*P*
Age (y), mean (SD)	68 ± 9	68 ± 9	68 ± 10	0.98[a]
Female, No. (%)	38 (25)	16 (21)	22 (29)	0.26[b]
Body mass index (kg/m^2), mean ± SD	22.6 ± 2.9	22.8 ± 3.0	22.3 ± 2.8	0.33[a]
Stage				0.44[b]
IA, No. (%)	75 (50)	37 (49)	38 (50)	
IB, No. (%)	36 (23)	16 (21)	20 (27)	
IIA, No. (%)	15 (10)	7 (9)	8 (11)	
IIB, No. (%)	10 (7)	5 (7)	5 (7)	
IIIA, No. (%)	15 (10)	11 (14)	4 (5)	
Pathology				0.10[b]
Squamous cell carcinoma	26 (17)	18 (24)	8 (11)	
Adenocarcinoma	122 (81)	57 (75)	65 (87)	
Large cell	3 (2)	1 (1)	2 (3)	
SNPs				
FokI CC/CT/TT	56/70/14	30/32/6	26/38/8	0.62[b]
BsmI AA/AG/GG	1/31/89	0/14/46	1/17/43	0.50[b]
Cdx2 GG/GA/AA	37/66/19	16/35/9	21/31/10	0.63[b]
ApaI GG/GT/TT	49/67/6	25/32/3	24/35/3	0.94[b]
TaqI TT/TC/CC	90/32/0	47/13/0	43/19/0	0.26[b]
DBP1 TT/TG/GG	61/49/12	31/22/7	30/27/5	0.66[b]
DBP2 CC/CA/AA	70/48/4	39/18/3	31/30/1	0.09[b]

[a]*P* value calculated by Student *t* test.
[b]*P* value calculated by the χ^2 test.

in the placebo group ($P = 0.14$). Serum calcium levels were not altered in both the vitamin D group ($P = 0.18$) and the placebo group ($P = 0.45$). Serum levels of 25(OH)D before taking the supplement were the highest in the DBP1 GG and DBP2 CC genotypes, medium in the DBP1 TT and DBP2 CC genotypes, and lowest in the DBP1 TT and DBP2 AA genotypes ($P = 0.01$).

Patients' characteristics

Patients' characteristics are shown in Table 1. The mean age was 68 years, and three-quarters of the patients were men. Early stages from IA to IIB and adenocarcinoma accounted for 90% and 81% of the study population, respectively. There were no significant differences in patients' characteristics between the vitamin D and placebo groups.

Effects of vitamin D or serum 25(OH)D levels on survival

Relapse and death occurred in 40 (28%) and 24 (17%) patients, respectively. Kaplan–Meier curves of RFS and OS were compared between the vitamin D group and the placebo group (Fig. 2A and B). The 5-year RFS of the vitamin D and placebo groups was 65% and 57%, respectively (HR, 1.15; 95% CI, 0.64–2.05; $P = 0.64$). The 5-year OS of the vitamin D and placebo groups was 76% and 78%, respectively (HR, 1.22; 95% CI, 0.54–2.79; $P = 0.63$).

The median and mean 25(OH)D levels before taking the supplement were 19 and 20.7 ng/mL, respectively. RFS and OS were compared between the lower (<20 ng/mL) and the higher (≥20 ng/mL) 25(OH)D groups (Fig. 2C and D). Patients with low 25(OH)D showed significantly poorer OS than patients with higher 25(OH)D ($P = 0.03$), which remained significant even after adjustment by early stage, adenocarcinoma, and vitamin D supplementation (HR, 0.37; 95% CI, 0.15–0.95; $P = 0.04$). However, when RFS and OS were compared between the 25(OH)D <30 and ≥30 ng/mL groups, there were no significant differences (Supplementary Fig. S1).

Subgroup analyses

By restricting the analysis to the subgroup of adenocarcinoma or adenocarcinoma plus early stage, RFS and OS curves were drawn (Supplementary Fig. S2). There was a clear trend to better survival in the vitamin D group than in the placebo group, although the differences were not significant.

The subgroup of adenocarcinoma and early stage was further divided into low 25(OH)D (Fig. 3A and B) and high 25(OH)D (Fig. 3C and D). In the subgroup of early-stage adenocarcinoma and low 25(OH)D, 5-year RFS in the vitamin D and placebo groups was 86% and 50%, respectively (log-rank test: $P = 0.04$; HR, 0.23; 95% CI, 0.05–1.05; $P = 0.06$). Similarly, 5-year OS in the vitamin D and placebo groups was 91% and 48%, respectively (log-rank test: $P = 0.02$; HR, 0.13; 95% CI, 0.02–0.99; $P = 0.049$).

Effects of SNPs on survivals

RFS and OS were compared among three kinds of genotypes in each SNP: Fok*I* (Supplementary Fig. S3); Bsm*I* (Supplementary Fig. S4), CDX2 (Supplementary Fig. S5), Taq*I* (Supplementary Fig. S6), Apa*I* (Supplementary Fig. S7), DBP1 (Supplementary Fig. S8), and DBP2 (Supplementary Fig. S9). RFS and OS were significantly different among three kinds of DBP1 genotypes (Supplementary Fig. S8A and S8B).

However, RFS and OS of patients with DBP1 TT were better than those of DBP1 GG/TG; the 5-year RFS in the DBP1 TT and DBP1 TG/GG genotypes was 73% and 33%, respectively (log-rank test: $P = 0.03$; HR, 0.50; 95% CI, 0.27–0.94; $P = 0.03$; Fig. 4A); similarly, the 5-year OS in the DBP1 TT and DBP1 TG/GG genotypes was 86% and 58%, respectively (log-rank test: $P = 0.004$; HR, 0.27; 95% CI, 0.10–0.69; $P = 0.007$; Fig. 4B). This finding, that patients with DBP1 TT genotype had longer RFS and OS than those with DBP1 TG/GG, remained significant even after adjustment by early stage, adenocarcinoma, low 25(OH)D, and vitamin D supplementation (HR, 0.51; 95% CI, 0.26–0.99; $P = 0.045$) and (HR, 0.21; 95% CI, 0.07–0.59; $P = 0.003$), respectively.

Regarding CDX2 genotypes, although there was no significant difference in 5-year RFS between the CDX2 AA/AG and GG genotypes (Fig. 4C), the 5-year OS of the CDX2 AA/AG and GG

Published OnlineFirst July 17, 2018; DOI: 10.1158/1078-0432.CCR-18-0483

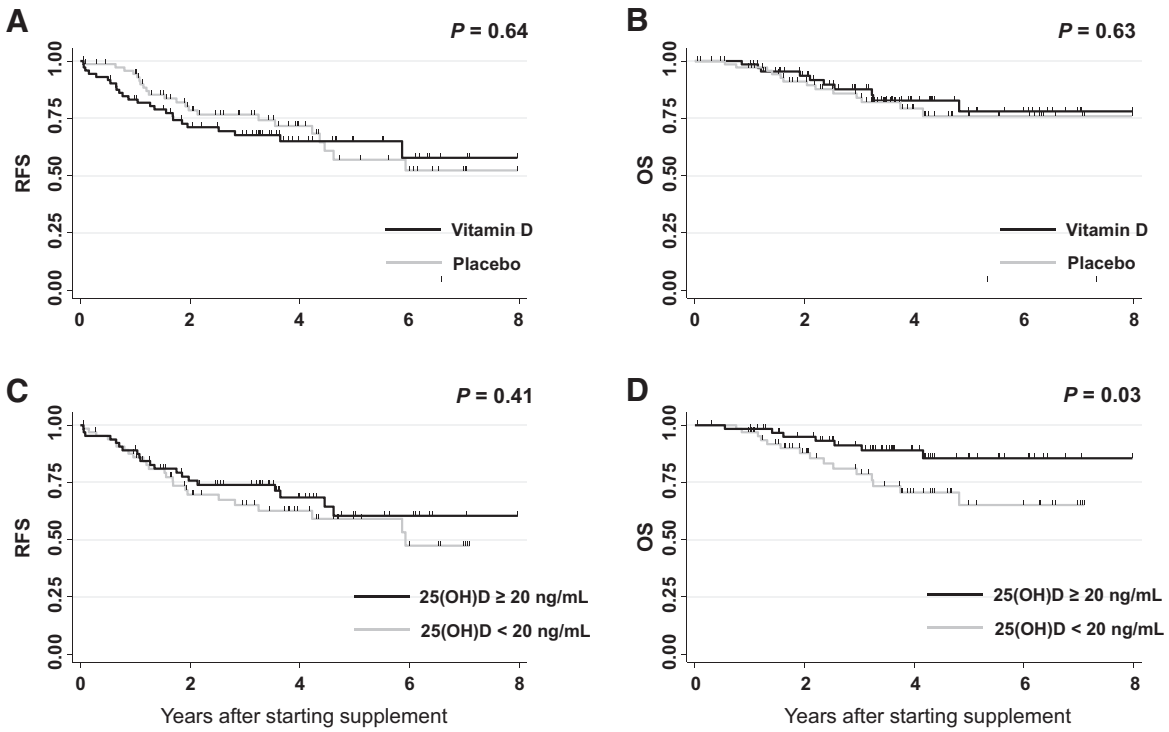

Figure 2.

Kaplan–Meier curves of patients with NSCLC years after starting supplement. *P* values were calculated by the log-rank test. **A,** Relapse-free survival curves of patients taking vitamin D (black line) versus those taking placebo (gray line). **B,** Overall survival curves of patients taking vitamin D (black line) versus those taking placebo (gray line). **C,** Relapse-free survival curves of patients with high 25(OH)D (≥20 ng/mL; black line) versus those with low 25(OH)D (<20 ng/mL; gray line) before taking supplements. **D,** Overall survival curves of patients with high 25(OH)D (≥20 ng/mL; black line) versus those with low 25(OH)D (<20 ng/mL; gray line) before taking supplements.

genotypes was 81% and 59%, respectively (log-rank test: $P = 0.04$; HR, 0.41; 95% CI, 0.18–0.97; $P = 0.04$; Fig. 4D). This finding, that patients with CDX2 AA/AG genotypes had longer OS than those with CDX2 GG genotypes, remained significant even after adjustment by early stage, adenocarcinoma, low 25(OH)D, and vitamin D supplementation (HR, 0.39; 95% CI, 0.16–0.97; $P = 0.04$).

Regarding CDX2 genotypes, the model was further stratified by low versus high 25(OH)D. In the low 25(OH)D subgroup, again, the 5-year RFS tended to be worse in GG compared with CDX2 AA/AG and genotypes, with no significant difference (Fig. 5A). On the other hand, the 5-year OS of the CDX2 AA/AG and GG genotypes was 78% and 27%, respectively (log-rank test: $P = 0.004$; HR, 0.24; 95% CI, 0.08–0.68; $P = 0.008$; Fig. 5B), which remained significant even after adjustment by early stage, adenocarcinoma, and vitamin D supplementation (HR, 0.22; 95% CI, 0.07–0.72; $P = 0.01$). On the other hand, in the high 25(OH)D subgroup, both 5-year RFS (Fig. 5C) and 5-year OS (Fig. 5D) were not significantly different between the CDX2 AA/AG and GG genotypes.

Safety

After finishing the supplements, there were no more cases of urinary stones, hypercalcemia, and serious adverse events in the vitamin D group than in the placebo group.

Discussion

In this RCT, daily supplementation with 1,200 IU of vitamin D for 12 months did not improve either RFS or OS of patients with NSCLC in the total study population including advanced stages and squamous cell carcinoma or large cell carcinoma. Thus, vitamin D was considered not likely to improve the survival of all patients with NSCLC, although the sample size might be too small to detect a significant difference.

Patients with 25(OH)D lower than 20 ng/mL showed significantly shorter OS than those with higher 25(OH)D, independent of intervention. In the current study, when the analysis was restricted to the subgroup of patients with early adenocarcinoma with lower 25(OH)D, vitamin D supplementation significantly improved RFS and OS. In previous cohort studies, higher 25(OH)D did not show an association with advanced stage (10, 19), but it did show an association with early stages (9). Anic and colleagues showed that patients with lung adenocarcinoma with higher 25(OH)D, but not patients with squamous cell carcinoma, tended to have better survival than those with lower 25(OH)D (20), which is consistent with the results of the current study. Moreover, serum 25(OH)D levels were not associated with the risk of lung cancer in general or in squamous cell carcinoma of the lung (21). Moreover, Kim and colleagues reported that 30% of patients with lung adenocarcinoma had high VDR mRNA levels, and these were correlated with longer survival (22). On the other hand, the same study group demonstrated that overexpression of

Published OnlineFirst July 17, 2018; DOI: 10.1158/1078-0432.CCR-18-0483

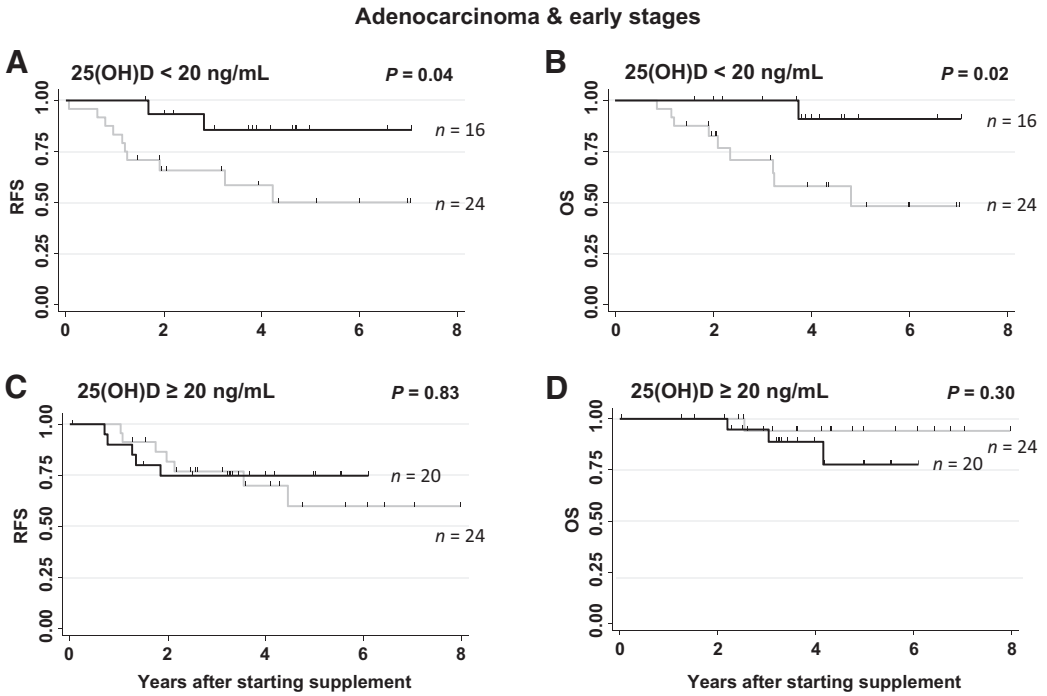

Figure 3.

Kaplan–Meier curves of patients restricted only to early-stage adenocarcinoma and further stratified by low 25(OH)D levels (<20 ng/mL; $n = 40$) or high 25(OH)D levels (≥20 ng/mL). *P* values were calculated by the log-rank test. **A,** Relapse-free survival curves of patients taking vitamin D (black line) versus those taking placebo (gray line) in early-stage adenocarcinoma with low 25(OH)D. **B,** Overall survival curves of patients taking vitamin D (black line) versus those taking placebo (gray line) in early-stage adenocarcinoma with low 25(OH)D. **C,** Relapse-free survival curves of patients taking vitamin D (black line) versus those taking placebo (gray line) in early-stage adenocarcinoma with high 25(OH)D. **D,** Overall survival curves of patients taking vitamin D (black line) versus those taking placebo (gray line) in early-stage adenocarcinoma with high 25(OH)D.

CYP24A1, which inactivates $1,25(OH)_2D$, is associated with poorer survival in lung adenocarcinoma (23). Because this trial was stopped earlier than planned at the interim analysis, the sample size was too small to perform subgroup analyses with sufficient power, especially in the subgroup with advanced stages ($n = 15$), squamous cell carcinoma ($n = 26$), or large cell carcinoma ($n = 3$). In addition, the number of patients with 25(OH)D ≥ 30(ng/mL) was also too small ($n = 20$) to show improved survivals depending on serum levels of 25(OH)D before supplementation. Thus, the results of subgroup analyses require further study.

Patients with the DBP1 TT genotype had significantly better survival than those with DBP1 TG/GG genotypes, which was not predicted. DBP binds to 85% to 90% of serum 25(OH)D (24), markedly prolongs the serum half-life of 25(OH)D (25), and is pivotal for renal handling of 25(OH)D and endocrine synthesis of $1,25(OH)_2D$ through megalin (26). However, DBP only transports 25(OH)D, but it does not facilitate uptake of 25(OH)D into target cells not expressing megalin (27), such as cancer cells. Thus, 25(OH)D unbound to DBP, so called bioavailable 25(OH)D, is hypothesized to drive many of the nonrenal actions of 25(OH)D (28). In fact, Powe and colleagues demonstrated that black Americans more frequently have DBP1 T allele and lower DBP and lower 25(OH)D serum levels than white Americans, resulting in almost equivalent levels of bioavailable 25(OH)D between black and white Americans and rather higher bone mineral density in black than in white Americans (29). The DBP1 TT genotype has the lowest affinity to 25(OH)D, probably resulting in higher levels of bioavailable 25(OH)D to cancer cells, which may thus improve patients' survival.

Patients with CDX2 AA/AG genotypes showed significantly better OS than those with the CDX2 GG genotype, a finding also reported by Zhou and colleagues (8). As a transcriptional factor, CDX2 binds to the VDR promoter region to regulate transcription of VDR. Of interest, the G-allele of CDX2 VDR SNPs was reported to have 70% less activity than the A-allele (30). Thus, patients with CDX2 AA/AG genotypes may have more VDR expression than those with the CDX2 GG genotype and a better prognosis, which was enhanced in the subgroup with low 25(OH)D, but not in that with high 25(OH)D. Patients with CDX2 GG may have less VDR expression, and low 25(OH)D may further hamper the anticancer effects of vitamin D.

The strength of the current study is that it is the first randomized, double-blind, placebo-controlled trial to examine whether vitamin D supplementation has an effect on the prognosis of patients with NSCLC, with follow-up of up to 8 years. However, there are several limitations to this study. First, the RCT was stopped based on the results of the interim analysis. Consequently, the number of randomized patients was only 155. Although there were significant differences in the subgroup, *P* values were 0.04 for RFS and 0.02 for OS in patients with early-stage lung adenocarcinoma with lower 25(OH)D level, which could still have occurred by chance. In addition, among subgroups

Published OnlineFirst July 17, 2018; DOI: 10.1158/1078-0432.CCR-18-0483

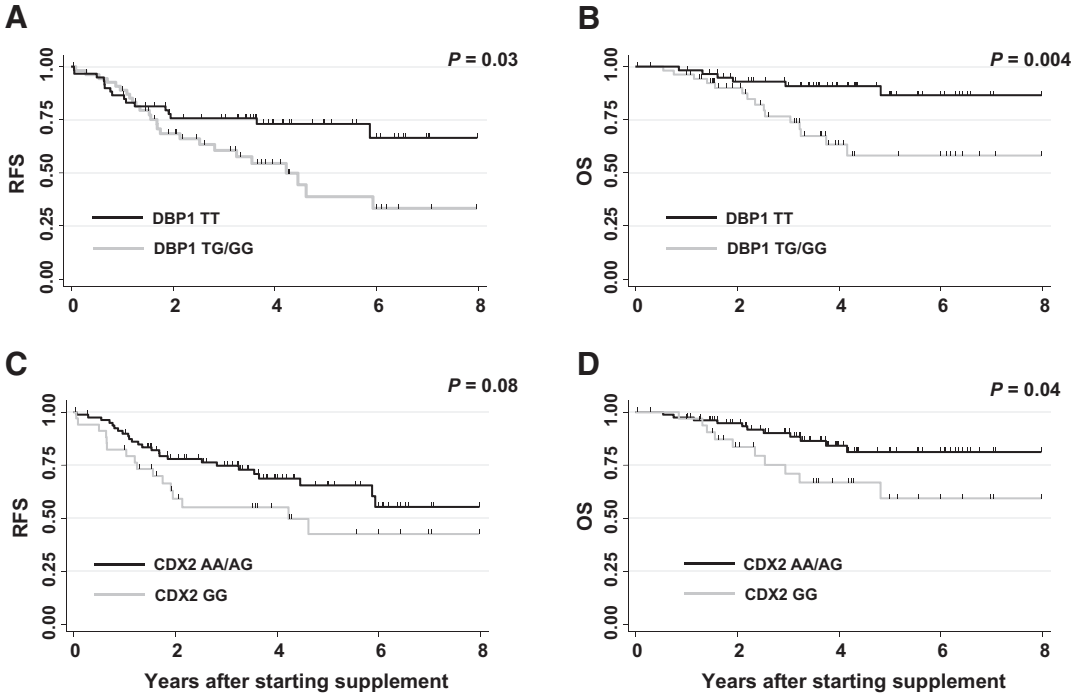

Figure 4.

Kaplan–Meier curves of the total study population without restriction or stratification. *P* values were calculated by the log-rank test. **A,** Relapse-free survival curves of patients with DBP1 TT genotype (black line) versus those with DBP1 GG/TG genotypes (gray line). **B,** Overall survival curves of patients with DBP1 TT genotype (black line) versus those with DBP1 GG/TG genotypes (gray line). **C,** Relapse-free survival curves of patients with CDX2 AA/AG genotypes (black line) versus those with CDX2 GG genotype (gray line). **D,** Overall survival curves of patients with CDX2 AA/AG genotypes (black line) versus those with CDX2 GG genotype (gray line).

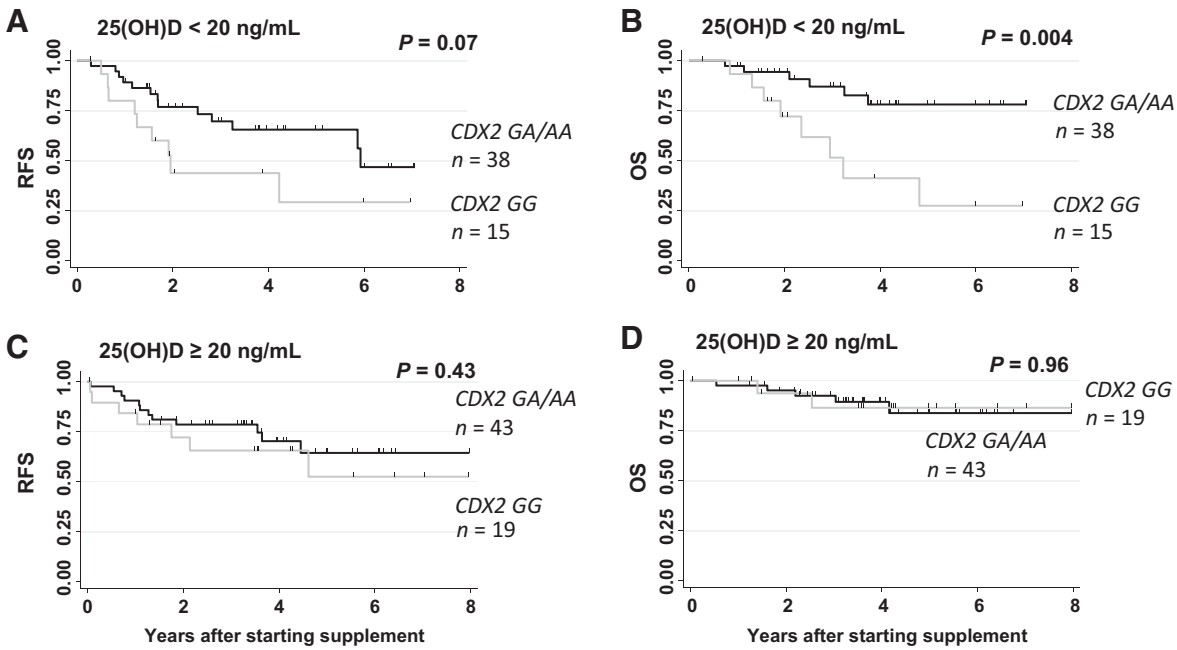

Figure 5.

Kaplan–Meier curves of all study population stratified by low 25(OH)D or high 25(OH)D and CDX2 genotypes. **A,** Relapse-free survival curves of patients with 25(OH)D < 20 ng/mL and with CDX2 AA/AG genotypes (black line) versus those with CDX2 GG genotype (gray line). **B,** Overall survival curves of patients with 25(OH)D < 20 ng/mL and with CDX2 AA/AG genotypes (black line) versus those with CDX2 GG genotype (gray line). **C,** Relapse-free survival curves of patients with 25(OH)D ≥ 20 ng/mL and with CDX2 AA/AG genotypes (black line) versus those with CDX2 GG genotype (gray line). **D,** Overall survival curves of patients with 25(OH)D ≥ 20 ng/mL and with CDX2 AA/AG genotypes (black line) versus those with CDX2 GG genotype (gray line).

Published OnlineFirst July 17, 2018; DOI: 10.1158/1078-0432.CCR-18-0483

stratified by the genotypes or by genotype-plasma 25(OH)D, the small subset numbers may also have resulted in some chance findings. Moreover, the sample size was too small to calculate *P* values for interactions within the subgroups. Second, vitamin D supplementation was used for only 12 months, with a dosage of 1,200 IU/day in this trial. Different regimens, including higher doses and longer durations, may lead to different findings. Third, in this study population, most patients had early stage and adenocarcinoma. On the other hand, advanced stages and SCC were fewer in number, and, thus, the effects of vitamin D on these subpopulations may be difficult to evaluate.

In conclusion, a randomized, double-blind, placebo-controlled trial was performed in patients with NSCLC and found that (i) vitamin D supplementation did not improve RFS and OS in the total study population; (ii) patients with high 25(OH)D (≥20 ng/mL) before taking the supplement showed better OS than those with low 25(OH)D (<20 ng/mL); (iii) in restricting the analysis to the subgroup with early-stage adenocarcinoma with low 25(OH)D, the vitamin D group showed significantly better 5-year RFS and OS than the placebo group; and (iv) among the examined polymorphisms, 5-year RFS and OS were better in patients with DBP1 TT than in those with TG/GG genotypes, as well as in patients with CDX2 AA/AG than with GG genotypes, both of which remained significant even after adjustment by stage, adenocarcinoma, low 25(OH)D, and vitamin D supplementation.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: T. Akiba, M. Urashima
Development of methodology: T. Akiba, M. Urashima
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): T. Akiba, T. Morikawa, M. Odaka, T. Nakada, N. Kamiya, M. Yamashita, M. Yabe, T. Inagaki, H. Asano, S. Mori, Y. Tsukamoto
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M. Urashima
Writing, review, and/or revision of the manuscript: M. Urashima
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): T. Morikawa, T. Inagaki
Study supervision: T. Akiba, T. Morikawa, M. Odaka, T. Nakada, N. Kamiya, M. Yamashita, M. Yabe, T. Inagaki, H. Asano, S. Mori, Y. Tsukamoto, M. Urashima

Acknowledgments

The authors would like to thank the patients for participating in this research project. They would also like to thank Chikako Sakanashi for technical support with PCR analysis and Naomi Sasagawa and Haruka Wada for data monitoring. This research was supported by the Ministry of Education, Culture, Sports, Science, and Technology in the Japan-Supported Program for the Strategic Research Foundation at Private Universities and by JSPH KAKENHI grant number: 22501060.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received February 9, 2018; revised April 3, 2018; accepted May 14, 2018; published first July 17, 2018.

References

1. Allemani C, Weir HK, Carreira H, Harewood R, Spika D, Wang XS, et al. Global surveillance of cancer survival 1995–2009: analysis of individual data for 25,676,887 patients from 279 population-based registries in 67 countries (CONCORD-2). Lancet 2015;385:977–1010.
2. Borghaei H, Paz-Ares L, Horn L, Spigel DR, Steins M, Ready NE, et al. Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. N Engl J Med 2015;373:1627–39.
3. Reck M, Rodríguez-Abreu D, Robinson AG, Hui R, Csőszi T, Fülöp A, et al. Pembrolizumab versus chemotherapy for PD-L1-positive non-small-cell lung cancer. N Engl J Med 2016;375:1823–1833.
4. Carbone DP, Reck M, Paz-Ares L, Creelan B, Horn L, Steins M, et al. First-line nivolumab in stage IV or recurrent non-small-cell lung cancer. N Engl J Med 2017;376:2415–2426.
5. Antonia SJ, Villegas A, Daniel D, Vicente D, Murakami S, Hui R, et al. Durvalumab after chemoradiotherapy in stage III non-small-cell lung cancer. N Engl J Med 2017;377:1919–1929.
6. Holick MF. Vitamin D deficiency. N Engl J Med 2007;357:266–81.
7. Bikle D. Nonclassic actions of vitamin D. J Clin Endocrinol Metab 2009; 94:26–34
8. Zhou W, Suk R, Liu G, Park S, Neuberg DS, Wain JC, et al. Vitamin D is associated with improved survival in early-stage non-small cell lung cancer patients. Cancer Epidemiol Biomarkers Prev 2005;14:2303–9.
9. Zhou W, Heist RS, Liu G, Asomaning K, Neuberg DS, Hollis BW, et al. Circulating 25-hydroxyvitamin D levels predict survival in early-stage non-small-cell lung cancer patients. J Clin Oncol 2007;25:479–85.
10. Heist RS, Zhou W, Wang Z, Liu G, Neuberg D, Su L, et al. Circulating 25-hydroxyvitamin D, VDR polymorphisms, and survival in advanced non-small-cell lung cancer. J Clin Oncol 2008;26:5596–602.
11. Braun A, Bichlmaier R, Cleve H. Molecular analysis of the gene for the human vitamin-D-binding protein (group-specific component): allelic differences of the common genetic GC types. Hum Genet 1992;89:401–6.
12. Arnaud J, Constans J. Affinity differences for vitamin D metabolites associated with the genetic isoforms of the human serum carrier protein (DBP). Hum Genet 1993;92:183–8.
13. Lauridsen AL, Vestergaard P, Hermann AP, Brot C, Heickendorff L, Mosekilde L, et al. Plasma concentrations of 25-hydroxy-vitamin D and 1,25-dihydroxy-vitamin D are related to the phenotype of Gc (vitamin D-binding protein): a cross-sectional study on 595 early postmenopausal women. Calcif Tissue Int 2005;77:15–22.
14. Sinotte M, Diorio C, Bérubé S, Pollak M, Brisson J. Genetic polymorphisms of the vitamin D binding protein and plasma concentrations of 25-hydroxyvitamin D in premenopausal women. Am J Clin Nutr 2009;89: 634–40.
15. Suzuki M, Yoshioka M, Hashimoto M, Murakami M, Noya M, Takahashi D, et al. Randomized, double-blind, placebo-controlled trial of vitamin D supplementation in Parkinson disease. Am J Clin Nutr 2013;97: 1004–13.
16. Hollis BW, Kamerud JQ, Selvaag SR, Lorenz JD, Napoli JL. Determination of vitamin D status by radioimmunoassay with an 125I-labeled tracer. Clin Chem 1993;39:529–33.
17. Suzuki M, Yoshioka M, Hashimoto M, Murakami M, Kawasaki K, Noya M, et al. 25-hydroxyvitamin D, vitamin D receptor gene polymorphisms, and severity of Parkinson's disease. Mov Disord 2012;27:264–71.
18. Schulz KF, Grimes DA. Multiplicity in randomised trials II: subgroup and interim analyses. Lancet 2005;365:1657–61.
19. Vashi PG, Edwin P, Popiel B, Gupta D. The relationship between circulating 25-hydroxyvitamin D and survival in newly diagnosed advanced non-small-cell lung cancer. BMC Cancer 2015;15:1012.
20. Anic GM, Weinstein SJ, Mondul AM, Männistö S, Albanes D. Serum vitamin D, vitamin D binding protein, and lung cancer survival. Lung Cancer 2014;86:297–303.
21. Sun YQ, Langhammer A, Wu C, Skorpen F, Chen Y, Nilsen TIL, et al. Associations of serum 25-hydroxyvitamin D level with incidence of lung cancer and histologic types in Norwegian adults: a case-cohort analysis of the HUNT study. Eur J Epidemiol 2018;33:67–77.
22. Kim SH, Chen G, King AN, Jeon CK, Christensen PJ, Zhao L, et al. Characterization of vitamin D receptor (VDR) in lung adenocarcinoma. Lung Cancer 2012;77:265–71.

Published OnlineFirst July 17, 2018; DOI: 10.1158/1078-0432.CCR-18-0483

23. Chen G, Kim SH, King AN, Zhao L, Simpson RU, Christensen PJ, et al. CYP24A1 is an independent prognostic marker of survival in patients with lung adenocarcinoma. Clin Cancer Res 2011;17:817–26.
24. Bikle DD, Gee E, Halloran B, Kowalski MA, Ryzen E, Haddad JG. Assessment of the free fraction of 25-hydroxyvitamin D in serum and its regulation by albumin and the vitamin D-binding protein. J Clin Endocrinol Metab 1986;63:954–9.
25. Safadi FF, Thornton P, Magiera H, Hollis BW, Gentile M, Haddad JG, et al. Osteopathy and resistance to vitamin D toxicity in mice null for vitamin D binding protein. J Clin Invest 1999;103:239–51.
26. Nykjaer A, Dragun D, Walther D, Vorum H, Jacobsen C, Herz J, et al. An endocytic pathway essential for renal uptake and activation of the steroid 25-(OH) vitamin D3. Cell 1999;96:507–15.
27. Bikle DD, Gee E. Free, and not total, 1,25-dihydroxyvitamin D regulates 25-hydroxyvitamin D metabolism by keratinocytes. Endocrinology 1989;124:649–54.
28. Chun RF, Peercy BE, Orwoll ES, Nielson CM, Adams JS, Hewison M. Vitamin D and DBP: the free hormone hypothesis revisited. J Steroid Biochem Mol Biol 2014;144Pt A:132–7.
29. Powe CE, Evans MK, Wenger J, Zonderman AB, Berg AH, Nalls M, et al. Vitamin D-binding protein and vitamin D status of black Americans and white Americans. N Engl J Med 2013;369:1991–2000.
30. Arai H, Miyamoto KI, Yoshida M, Yamamoto H, Taketani Y, Morita K, et al. The polymorphism in the caudal-related homeodomain protein Cdx-2 binding element in the human vitamin D receptor gene. J Bone Miner Res 2001;16:1256–64.

AACR American Association for Cancer Research

Clinical Cancer Research

Vitamin D Supplementation and Survival of Patients with Non–small Cell Lung Cancer: A Randomized, Double-Blind, Placebo-Controlled Trial

Tadashi Akiba, Toshiaki Morikawa, Makoto Odaka, et al.

Clin Cancer Res Published OnlineFirst July 17, 2018.