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Effect of Vitamin D Supplementation on Relapse-Free Survival Among Patients With Digestive Tract Cancers
The AMATERASU Randomized Clinical Trial

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IMPORTANCE Randomized clinical trials of vitamin D supplementation for secondary prevention in patients with cancer are needed, given positive results of observational studies.

OBJECTIVE To determine whether postoperative vitamin D_3 supplementation can improve survival of patients with digestive tract cancers overall and in subgroups stratified by 25-hydroxyvitamin D (25[OH]D) levels.

DESIGN, SETTING, AND PARTICIPANTS The AMATERASU trial, a randomized, double-blind, placebo-controlled trial conducted at a single university hospital in Japan. Enrollment began in January 2010 and follow-up was completed in February 2018. Patients aged 30 to 90 years with cancers of the digestive tract from the esophagus to the rectum, stages I to III, were recruited. Of 439 eligible patients, 15 declined and 7 were excluded after operation.

INTERVENTIONS Patients were randomized to receive oral supplemental capsules of vitamin D (2000 IU/d; n = 251) or placebo (n = 166) from the first postoperative outpatient visit to until the end of the trial.

MAIN OUTCOMES AND MEASURES The primary outcome was relapse-free survival time to relapse or death. The secondary outcome was overall survival time to death due to any cause. Subgroups analyzed had baseline serum 25(OH)D levels of 0 to less than 20 ng/mL, 20 to 40 ng/mL, and greater than 40 ng/mL; because of small sample size for the highest-baseline-level group, interactions were tested only between the low- and middle-baseline-level groups.

RESULTS All 417 randomized patients (mean age, 66 years; male, 66%; esophageal cancer, 10%; gastric cancer, 42%; colorectal cancer, 48%) were included in the analyses. There was 99.8% follow-up over a median 3.5 (interquartile range, 2.3-5.3) years, with maximal follow-up of 7.6 years. Relapse or death occurred in 50 patients (20%) randomized to vitamin D and 43 patients (26%) randomized to placebo. Death occurred in 37 (15%) in the vitamin D group and 25 (15%) in the placebo group. The 5-year relapse-free survival was 77% with vitamin D vs 69% with placebo (hazard ratio [HR] for relapse or death, 0.76; 95% CI, 0.50-1.14; $P = .18$). The 5-year overall survival in the vitamin D vs placebo groups was 82% vs 81% (HR for death, 0.95; 95% CI, 0.57-1.57; $P = .83$). In the subgroup of patients with baseline serum 25(OH)D levels between 20 and 40 ng/mL, the 5-year relapse-free survival was 85% with vitamin D vs 71% with placebo (HR for relapse or death, 0.46; 95% CI, 0.24-0.86; $P = .02$; $P = .04$ for interaction). Fractures occurred in 3 patients (1.3%) in the vitamin D group and 5 (3.4%) in the placebo group. Urinary stones occurred in 2 patients (0.9%) in the vitamin D group and 0 in the placebo group.

CONCLUSIONS AND RELEVANCE Among patients with digestive tract cancer, vitamin D supplementation, compared with placebo, did not result in significant improvement in relapse-free survival at 5 years.

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Serum levels of 25-hydroxyvitamin D (25[OH]D), a precursor of activated vitamin D, increase in response to exposure to sunlight, a vitamin D–rich diet, or vitamin D supplementation. Cancer cells are believed to take up and activate 25(OH)D within the cell, which binds to the vitamin D receptor to regulate gene expression and consequently suppresses cancer growth.[1,2]

In 1989, the risk of colon cancer was estimated to be 70% lower in people with serum 25(OH)D levels of 20 ng/mL or more compared with those with levels less than 20 ng/mL.[3] In a cohort study, higher 25(OH)D levels were associated with lower total cancer incidence and lower total cancer mortality, particularly digestive system cancer mortality.[4] Similar results have been found in colorectal cancer.[5-7]

However, 2 randomized clinical trials (RCTs) using vitamin D and calcium to prevent cancer incidence generated conflicting conclusions: one concluded that supplementation with calcium and vitamin D (400 IU/d) for 7 years had no effect on the incidence of colorectal cancer in postmenopausal women,[8] whereas another demonstrated that cancer incidence was lower in postmenopausal women taking calcium plus vitamin D (1100 IU/d) than in those taking placebo.[9] However, these RCTs focused on primary prevention for participants without cancer; to our knowledge, no RCT of secondary prevention for relapse or death has been conducted.

Single-nucleotide polymorphisms (SNPs) of the vitamin D receptor have been associated with the prognosis of patients with cancer.[10-12] In addition, SNPs of vitamin D binding protein have been associated with 25(OH)D levels.[13,14] Therefore, a randomized, double-blind, placebo-controlled trial was conducted to assess whether vitamin D_3 supplementation can improve survival of patients with digestive tract cancers from the esophagus to the rectum after surgical resection. Subgroup analyses were also done based on serum 25(OH)D cutoff levels of 20 and 40 ng/mL as well as the presence of relevant SNPs.

Key Points

Question Does vitamin D supplementation improve survival among patients with digestive tract cancers?

Findings In this randomized clinical trial that included 417 patients with digestive tract cancers (from esophagus to rectum), the 5-year relapse-free survival rate for those randomized to vitamin D, 2000 IU/d, vs placebo was 77% vs 69%, a difference that was not statistically significant.

Meaning Vitamin D supplementation did not improve relapse-free survival among patients with digestive tract cancer.

Methods

Trial Design

This was a double-blind, placebo-controlled trial to compare the effects of vitamin D_3 supplementation, 2000 IU/d, or placebo at an allocation ratio of 3:2. Patients at the International University of Health and Welfare Hospital (Ohtawara, Tochigi prefecture, Japan) surgically treated for digestive tract cancer between the esophagus and the rectum are usually discharged 5 to 14 days after the operation and visit the outpatient clinic 1 to 2 weeks later if there are no major complications. Therefore, in this study, patients who did not meet exclusion criteria were randomized and started supplementation at the first outpatient visit between 2 and 4 weeks after operation. The trial protocol was approved by the ethics committee of the International University of Health and Welfare Hospital. Written informed consent was obtained from each patient who decided to participate before surgery. There was no external data and safety monitoring committee for this study. The full protocol for this trial is available in Supplement 1.

Participants

The inclusion criteria included a histopathological diagnosis of epithelial carcinoma of the digestive tract (esophagus, stomach, small intestine, colon, and rectum), clinical stages I to III; aged 30 to 90 years at entry; diagnosis and initial surgery at the International University of Health and Welfare Hospital; not taking vitamin D supplements or active vitamin D; and no history of urinary tract stones. The exclusion criteria comprised tumors that were not resectable by surgery, serious postoperative complications before starting supplementation, pathological diagnosis other than epithelial carcinoma (such as malignant lymphoma and sarcoma), and pathological stage 0 or IV. Collaborating surgeons preoperatively described the trial to eligible patients and their families at outpatient clinics and asked them to participate in the trial. All clinical data were collected at the International University of Health and Welfare Hospital and monitored at the Division of Molecular Epidemiology, Jikei University School of Medicine.

Randomization and Blinding

Computer-generated and centrally administered randomization used permuted blocks of 5. Participants were randomized in a 3:2 ratio without stratification. Increasing the likelihood of randomization to the vitamin D group was done in an effort to increase willingness to participate. With the exception of M.U. and the staff of the data monitoring center at the Jikei University School of Medicine, who prepared the bottles of vitamin D or placebo according to randomization assignments, all other surgeons, the clinical research coordinator, and participants at the International University of Health and Welfare Hospital were blinded to group assignment.

Intervention

At the first outpatient visit after surgery, enrolled patients were randomly assigned to receive either vitamin D_3 supplementation, 2000 IU/d, or placebo, and were asked to take the study medication from that day until the end of the trial. Both study medications were purchased from Zenyaku Pharmaceutical Co Ltd, Tokyo, Japan.

Outcomes

The primary outcome was relapse-free survival, defined as elapsed time from the date of randomization (ie, time from starting the study medication to the earliest date of cancer relapse or death due to any cause). Secondary outcomes comprised overall survival, defined as elapsed time from the date of randomization (ie, time from starting the study medication to the date of death due to any cause), as well as incidence of relapse, cancer-specific death, and noncancer death. Safety outcomes comprised

bone fractures, urinary stones, serious events requiring admission, and new (de novo) cancer arising in organs other than the site of the primary cancer after starting study medication.

Follow-up

Patients were periodically examined as outpatients by computed tomography, magnetic resonance imaging, positron emission tomography, and other procedures to exclude cancer relapse as required by a surgeon in charge. This generally occurred every month for the first 6 months, every 2 months for the second 6 months, and every 3 months thereafter until 5 years. After 5 years, follow-up continued to occur every 3 to 6 months depending on a patient's condition as judged by the surgeon in charge. A clinical research coordinator interviewed participants about self-reported adherence at every visit to the outpatient clinic of the International University of Health and Welfare Hospital. The coordinator sometimes made telephone calls to participants to confirm their health condition and adherence, and again confirmed their adherence when she provided a new bottle of trial medication every 6 months. Moreover, levels of 25(OH)D were measured annually in blood samples to determine changes in the vitamin D and placebo groups. Preoperative and postoperative chemotherapy was administered to patients with stage II and III esophageal cancer. Postoperative chemotherapy was administered to patients with stage II and III gastric cancer[15] and all patients with stage III colorectal cancer.[16] Local radiation or molecular-targeting therapy was combined with chemotherapy for selected patients with relapse. When vitamin D supplementation was medically required for conditions such as bone fracture or osteoporosis, trial supplementation was stopped, but the patients were followed up until the end of the study.

Vitamin D Measurements

Serum levels of 25(OH)D were measured using radioimmunoassay (SRL Inc, Hachioji, Tokyo, Japan) as previously described[17] and every year (within the same calendar month) after starting supplements. Using 19 serum samples obtained from a different cohort, blinded duplicates were tested for this 25(OH)D assay and the correlation coefficient was 0.92.

SNP Analyses of Vitamin D Receptor and Vitamin D Binding Protein

Analyses were conducted of particular SNPs associated with the vitamin D receptor. These included Fok*I* (rs2228570); Bsm*I* (rs1544410); CDX2 (rs11568820); Apa*I* (rs7976091); and Taq*I* (rs731236) because they have been reported to be associated with function of the vitamin D receptor.[18] Moreover, among SNPs of vitamin D binding protein, DBP1 (rs7041) and DBP2 (rs4588) were chosen because these SNPs are missense mutations that influence serum levels of 25(OH)D.[13,14] DNA was extracted from peripheral blood samples and stored at −80°C. DNA fragments were amplified by polymerase chain reaction. The SNPs were determined by direct sequencing, for which the detailed methods were described in a previous article[19] and in eAppendix 1 in Supplement 1.

Sample Size

When raw data from the cohort of 257 patients with colorectal cancer were studied at the Jikei University School of Medicine,[7] 5-year relapse-free survival in the highest quartile of 25(OH)D levels was calculated to be 13% better than in the lowest quartile. Considering approximate survival data for digestive tract cancer in Japan, the 5-year relapse-free survival rates would be 75% and 62% in the vitamin D and placebo groups, respectively, with a 2-side type I error of .05 and a power of 80%, assuming a 1% loss to follow-up. It was estimated that 400 patients with digestive tract cancers divided in a 3:2 ratio would be sufficient to detect this difference. Assuming that 80 patients per year could participate in this trial, the accrual period was estimated to be 5 years to enroll 400 participants. With final patient follow-up 2 years after enrollment, the total duration of the planned trial was 7 years.

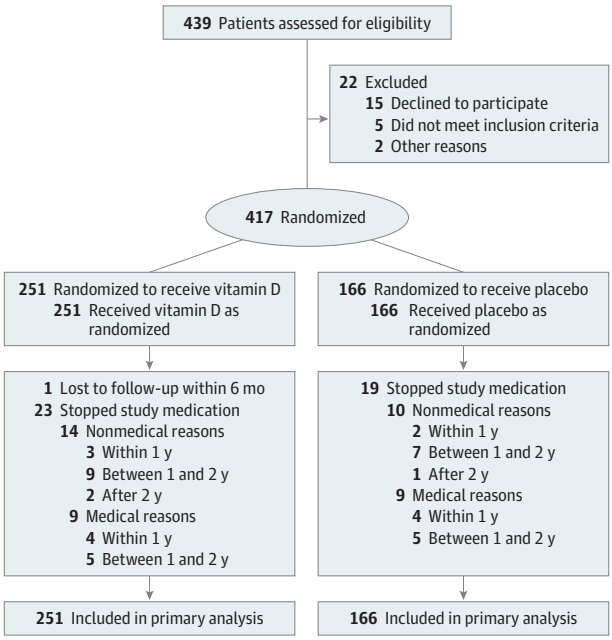
Statistical Analysis

All patients who underwent randomization were included in this analysis. Relapse- and death-related outcomes were assessed according to randomization group whether or not supplements were taken, whereas adverse events were assessed only in patients who continued to take the supplements (per protocol). The effects of vitamin D and placebo on risk of relapse or death and total deaths were estimated using Nelson-Aalen cumulative hazard curves for outcomes. A Cox proportional hazards model was used to determine hazard ratios (HRs) and 95% confidence intervals. Subgroups were prespecified according to 25(OH)D levels at baseline—low (<20 ng/mL), middle (20-40 ng/mL), or high (>40 ng/mL)—and by SNPs of vitamin D receptor and vitamin D binding protein. Patients with missing data for 25(OH)D levels at baseline were not included in the subgroup analyses. To clarify whether vitamin D supplementation significantly affected these subgroups, *P* values for interaction were analyzed based on a Cox regression model that included treatment allocation, baseline 25(OH)D group, and treatment allocation and baseline 25(OH)D group multiplied together as an interaction variable by 2-way interaction tests comparing the low and middle subgroups; the high-baseline-level subgroup was not included because of the small number of patients in that subgroup. Values with a 2-sided $P < .05$ were considered statistically significant. However, because of the potential for type I error due to multiple comparisons, findings for subgroup analyses should be interpreted as exploratory.

All data were analyzed using Stata version 14.0 (StataCorp). Annual interim analyses were planned after entry of 200 patients. Statistical significance at the interim analysis was set at $P<.001$ according to Peto stopping boundaries[20] because it uses constant but stringent stopping levels independent of the number of interim analyses that could not be exactly predicted.

There were a number of post hoc analyses. First, the proportional hazards assumption was tested using Schoenfeld residuals.[21] Second, to evaluate the effects of vitamin D supplementation on relapse, cumulative incidence functions were applied by considering patient deaths due to causes other than relapse as a competing risk; competing risk regression was performed using subdistribution HRs and 95% confidence intervals.[22] Third, because patients in the vitamin D group were older and had more stage I disease than in the placebo group, HRs were adjusted by age quartiles or stage I disease status. Fourth, multiple imputation was performed with a Markov chain Monte Carlo technique using multivariable normal regression repeated 50 times for missing 25(OH)D data using

Figure 1. Patient Flow Through the AMATERASU Trial



patient age and sex and serum levels of calcium, alkaline phosphatase, and parathyroid hormone.

Fifth, subgroup analyses for cancer site, stages, major pathology, sex, body mass index, and age were performed. Sixth, because levels of 25(OH)D were not increased among all patients randomized to vitamin D supplementation, participants were stratified by their mean 25(OH)D levels over the course of the trial, including at baseline and during annual blood sampling, independent of vitamin D supplementation (<20, 20 to <30, 30 to <40, 40 to <50, or ≥50 ng/mL). Nelson-Aalen cumulative hazard curves of relapse or death were drawn by these stratified groups (eFigure 1, A and B, and eTable 1 in Supplement 2). Seventh, changes in the median levels of serum 25(OH)D and calcium from baseline to 1 year in each group was analyzed using Wilcoxon signed rank test; change ratios at 1 year were compared between the vitamin D and placebo groups using the Mann-Whitney test.

Results

Study Population

There were 3 interim analyses, but the trial was continued until the number of entries exceeded 400 because the stopping rule was not met. **Figure 1** shows the flow of the 417 patients with digestive tract cancers who were randomized to receive vitamin D supplementation (n = 251 [60%]) or placebo (n = 166 [40%]) between January 2010 and April 2016. At a median 23.5 (interquartile range [IQR], 13-44) days after operation, participants were randomized and started study medication. All surviving patients were followed up until February 2018 except 1 patient who was lost to follow-up at 1 month (follow-up rate, 99.8%). The number of participants who stopped trial medication for nonmedical reasons, including the 1 patient lost to follow-up, was 15 (6.0%) in the vitamin D group and 10 (6.0%) in the placebo group (Figure 1). In addition, 9 (3.6%) in the vitamin D group and 9 (5.4%) in the placebo group stopped trial medication for medical reasons such as swallowing disorder, fracture and osteoporosis, and urinary stones. Therefore, 10.3% of participants stopped taking study medication before censoring, but all 417 patients were included in the efficacy analyses.

The median and maximum follow-up periods respectively were 3.5 (IQR, 2.3-5.4) years and 7.6 years in the vitamin D group and 3.5 (IQR, 2.3-5.0) years and 7.4 years in the placebo group. **Table 1** shows the characteristics of the patients, all of whom were Japanese. Of the 417 participants, 34% were women. The median age was 66 years and the median body mass index was 22. Percentages of cancer sites were as follows: esophagus, 9.6%; stomach, 41.7%; small bowel, 0.5%; and colorectal, 48.2%. Disease stages were I, II, and III in 44%, 26%, and 30% of patients, respectively. The median age of patients was 67 (IQR, 61-75) years in the vitamin D group and 64 (IQR, 58-71) years in the placebo group. The percentage of patients with stage I disease was 46% in the vitamin D group and 40% in the placebo group.

Effects of Vitamin D Supplementation

Relapse or death occurred in 50 patients (20%) taking vitamin D and 43 patients (26%) taking placebo. Among the participants, 37 (15%) of 251 in the vitamin D group and 25 (15%) of 166 in the placebo group died. Relapse, cancer-specific death, and noncancer death respectively occurred in 41 (16%), 27 (11%), and 10 (4%) patients in the vitamin D group and in 36 (22%), 16 (10%), and 9 (5%) patients in the placebo group.

The primary outcome of 5-year relapse-free survival in the vitamin D group vs placebo group was 77% vs 69%. Vitamin D supplementation did not significantly reduce risk of relapse or death compared with placebo (HR, 0.76; 95% CI, 0.50-1.14; P = .18) (**Figure 2**A). The secondary outcome of 5-year overall survival was 82% in the vitamin D group vs 81% in the placebo group (HR for death, 0.95; 95% CI, 0.57-1.57; P = .83) (Figure 2B). Hazard ratios for cancer-specific death and noncancer death are shown in **Table 2**.

Associations With Prespecified Subgroups: 25(OH)D Levels and SNPs

Patients were divided into prespecified subgroups according to low (<20 ng/mL [n = 173]), middle (20-40 ng/mL [n = 232]), or high (>40 ng/mL [n = 5]) serum levels of 25(OH)D at baseline. Because of the small number of patients in the high-baseline-level subgroup, further analyses within this subgroup were not conducted. Vitamin D supplementation increased median 25(OH)D levels from 26.5 (IQR, 23-30) ng/mL to 45 (IQR, 36-57) ng/mL after 1 year in the middle-baseline-level subgroup, whereas they increased from 16 (IQR, 13-18) ng/mL to 36 (IQR, 27-47) ng/mL in the low-baseline-level subgroup (eFigure 2 in Supplement 2).

In the middle-baseline-level subgroup, 5-year relapse-free survival was significantly higher in the vitamin D group than in the placebo group (85% vs 71%; HR for relapse or death, 0.46; 95% CI, 0.24-0.86; P = .02) (**Figure 3**A). There was no significant difference for relapse-free survival in the low-baseline-level subgroup (HR, 1.15; 95% CI, 0.65-2.05) (Figure 3B). There

Table 1. Participant Characteristics

Characteristics	No. (%) of Participants[a]	
	Vitamin D (n = 251)	Placebo (n = 166)
Male	173 (69)	103 (62)
Female	78 (31)	63 (38)
Age quartile, y		
35-59	51 (20)	50 (30)
60-65	55 (22)	41 (25)
66-73	63 (25)	48 (29)
74-90	82 (33)	27 (16)
Body mass index quartile[b]		
15.0-19.7	63 (25)	36 (22)
19.8-21.8	62 (25)	43 (26)
21.9-23.7	59 (24)	45 (27)
23.8-37.3	65 (26)	41 (25)
History of other cancers	9 (4)	7 (4)
Comorbid conditions		
Hypertension	103 (41)	58 (35)
Diabetes mellitus	44 (18)	24 (14)
Endocrine disease	33 (13)	18 (11)
Cardiovascular disease	22 (9)	9 (5)
Chronic kidney disease	5 (2)	1 (1)
Asthma	3 (1.2)	0 (0.0)
Orthopedic disease	1 (0.4)	1 (0.6)
Site of cancer		
Esophagus	22 (9)	18 (11)
Stomach	106 (42)	68 (41)
Small bowel	1 (0.4)	1 (0.6)
Colorectal	122 (49)	79 (48)
Cancer stage		
I	115 (46)	67 (40)
II	63 (25)	48 (29)
III	73 (29)	51 (31)
Pathology		
Adenocarcinoma	226 (90)	147 (88)
Squamous cell carcinoma	22 (9)	16 (10)
Other[c]	3 (1)	3 (2)
Baseline 25-hydroxyvitamin D level, ng/mL		
Low: <20	102 (41)	71 (44)
Middle: 20-40	142 (58)	90 (56)
High: >40	4 (1.6)	1 (0.6)
Adjuvant chemotherapy	88 (35)	60 (36)
Single-nucleotide polymorphism		
*Fok*I		
CC	92 (38)	57 (36)
CT	117 (48)	75 (48)
TT	36 (15)	25 (16)
*Bsm*I		
AA	14 (6)	8 (5)
AG	42 (18)	23 (15)
GG	175 (76)	119 (79)
Cdx2		
GG	89 (39)	49 (33)
GA	103 (45)	77 (51)
AA	38 (17)	24 (16)
*Apa*I		
GG	96 (42)	69 (46)
GT	104 (45)	61 (40)
TT	31 (13)	20 (13)
*Taq*I		
TT	172 (74)	115 (77)
TC	54 (23)	31 (21)
CC	5 (2)	4 (3)
DBP1		
TT	134 (58)	82 (55)
TG	87 (38)	57 (39)
GG	10 (4)	9 (6)
DBP2		
CC	115 (50)	81 (54)
CA	91 (39)	58 (39)
AA	25 (11)	11 (7)

[a] Percentages may not sum to 100% because of rounding.

[b] Calculated as weight in kilograms divided by height in meters squared.

[c] Neuroendocrine tumor (n=4); adenoid cystic carcinoma (n=1); unclassified (n=1).

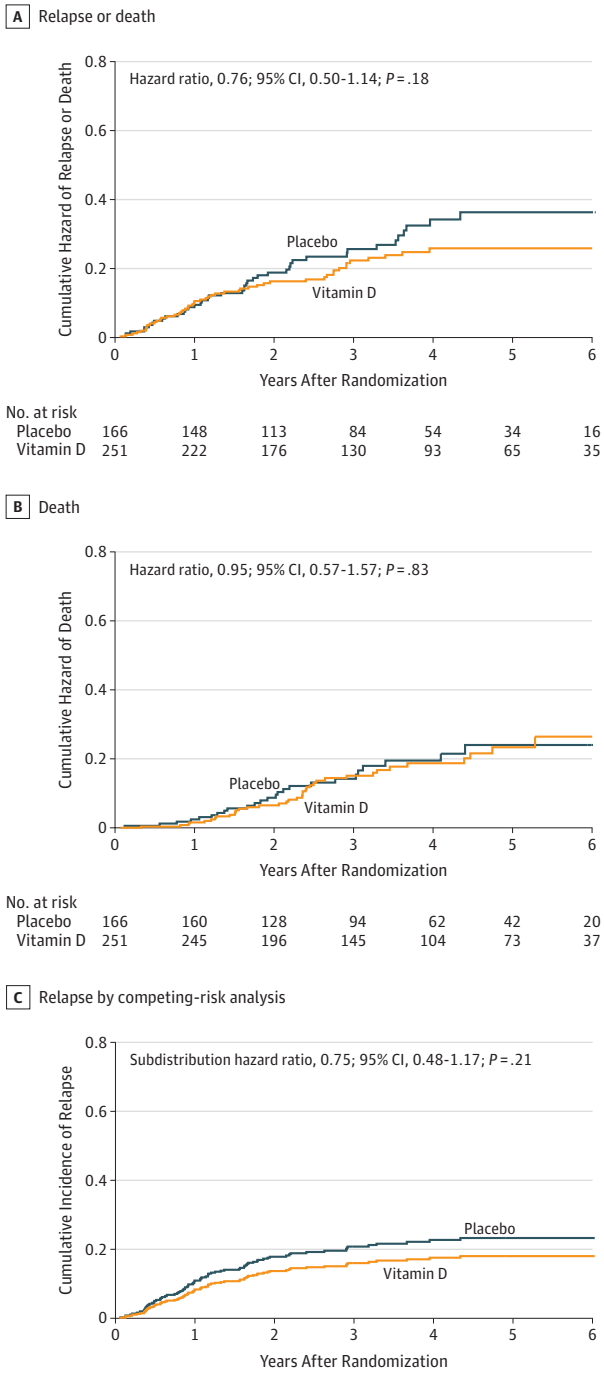
was a significant 2-way interaction between the middle and low subgroups (P = .04 for interaction). Overall survival was not significantly different between the vitamin D and placebo groups in both the middle subgroup (HR for death, 0.60; 95% CI, 0.28-1.30) (Figure 3C) and the low subgroup (HR for death, 1.36; 95% CI, 0.66-2.81) (Figure 3D) (P = .13 for interaction). Subgroup analyses between the middle and low subgroups for cancer-specific death and noncancer death are shown in Table 2.

Significant associations were not seen between subgroups of SNPs (eFigure 3, A-U, in Supplement 2).

Post Hoc Analyses

Results of the proportional hazards assumption test were not significant, supporting validity of the Cox proportional hazards

Figure 2. Effect of Vitamin D Supplementation on Outcomes



Nelson-Aalen cumulative hazard curves are shown for (A) relapse or death and (B) total deaths. In panel C, the cumulative incidence of relapse is compared between the vitamin D and placebo groups by considering patient deaths due to causes other than relapse as the competing risk. Median observation times for relapse or death were, for placebo, 3.0 (interquartile range [IQR], 1.9-4.5) years, and for vitamin D, 3.3 (IQR, 1.9-5.3) years; for total deaths, for placebo, 3.5 (IQR, 2.3-5.0) years, and for vitamin D, 3.5 (IQR, 2.3-5.4) years; and for relapse by competing-risk analysis, for placebo, 3.0 (IQR, 1.9-4.5) years, and for vitamin D, 3.3 (IQR, 1.9-5.3) years.

models used in the analyses. There were 7 patients with missing 25(OH)D levels, and results using multiple imputation were consistent with the primary results (eTable 2 in Supplement 2).

Using methods for competing-risk analysis, the cumulative incidence of relapse was compared, and there was no significant difference (subdistribution HR, 0.75; 95% CI, 0.48-1.17; P = .21) (Figure 2C). In the subgroup with middle baseline levels of 25(OH)D, the cumulative incidence of relapse was significantly lower in the vitamin D group than in the placebo group (subdistribution HR, 0.44; 95% CI, 0.21-0.89; P = .02) (Table 2). There was no significant difference for the cumulative incidence of relapse in the low-baseline-level subgroup (subdistribution HR, 1.18; 95% CI, 0.64-2.19). There was a significant 2-way interaction between the middle- and low-baseline-level subgroups (P = .04 for interaction).

Because patients were older in the vitamin D group than in the placebo group (Table 1), HRs were adjusted by age quartile. In this adjusted analysis, the cumulative hazard of relapse or death was significantly less in the vitamin D group compared with the placebo group, with an adjusted HR of 0.66 (95% CI, 0.43-0.99; P = .048). In contrast, the cumulative hazard of death was not significantly different, with an adjusted HR of 0.81 (95% CI, 0.48-1.36; P = .42). Patients with stage I cancer were more prevalent in the vitamin D group; analyses adjusted for stage I disease status did not show any significant differences.

Interactions between vitamin D supplementation and the following subgroups were examined, but no significant associations were found: men vs women (eFigure 4, A and B, in Supplement 2); age 65 years or younger vs older than 65 years (eFigure 5, A and B, in Supplement 2); body mass index less than 25 vs 25 or higher (eFigure 6, A and B, in Supplement 2); site of cancer: esophageal, gastric, or colorectal (eFigure 7, A-C, in Supplement 2); disease stage: I, II, or III (eFigure 8, A-C, in Supplement 2); and adenocarcinoma vs nonadenocarcinoma (eFigure 9, A and B, in Supplement 2).

Median 25(OH)D levels increased significantly in the vitamin D group, from 21 (IQR, 16-27) ng/mL to 41 (IQR, 33-55) ng/mL (P < .001), but did not change significantly in the placebo group, from 21 (IQR, 15-27) ng/mL to 20 (IQR, 15-27) ng/mL (P = .91). The change ratio of 25(OH)D level was 87% in the vitamin D group vs 0% in the placebo group 1 year after starting study medication (P < .001). Levels of 25(OH)D in the vitamin D group remained high over 4 years of trial follow-up, while those in the placebo group remained low (eFigure 2 in Supplement 2). Analyses by mean 25(OH)D level during the trial are shown in eTable 1 and eFigure 1, A and B. Changes in median serum calcium levels were not significantly different in the vitamin D group (from 9.3 [IQR, 8.9-9.6] mg/dL to 9.3 [IQR, 9.1-9.6] mg/dL; P = .09) or in the placebo group (from 9.3 [IQR, 9.0-9.6] mg/dL to 9.3 [IQR, 9.1-9.5] mg/dL; P = .44). The change ratio of calcium was 0% in both the vitamin D and placebo groups (P = .10).

Adverse Events

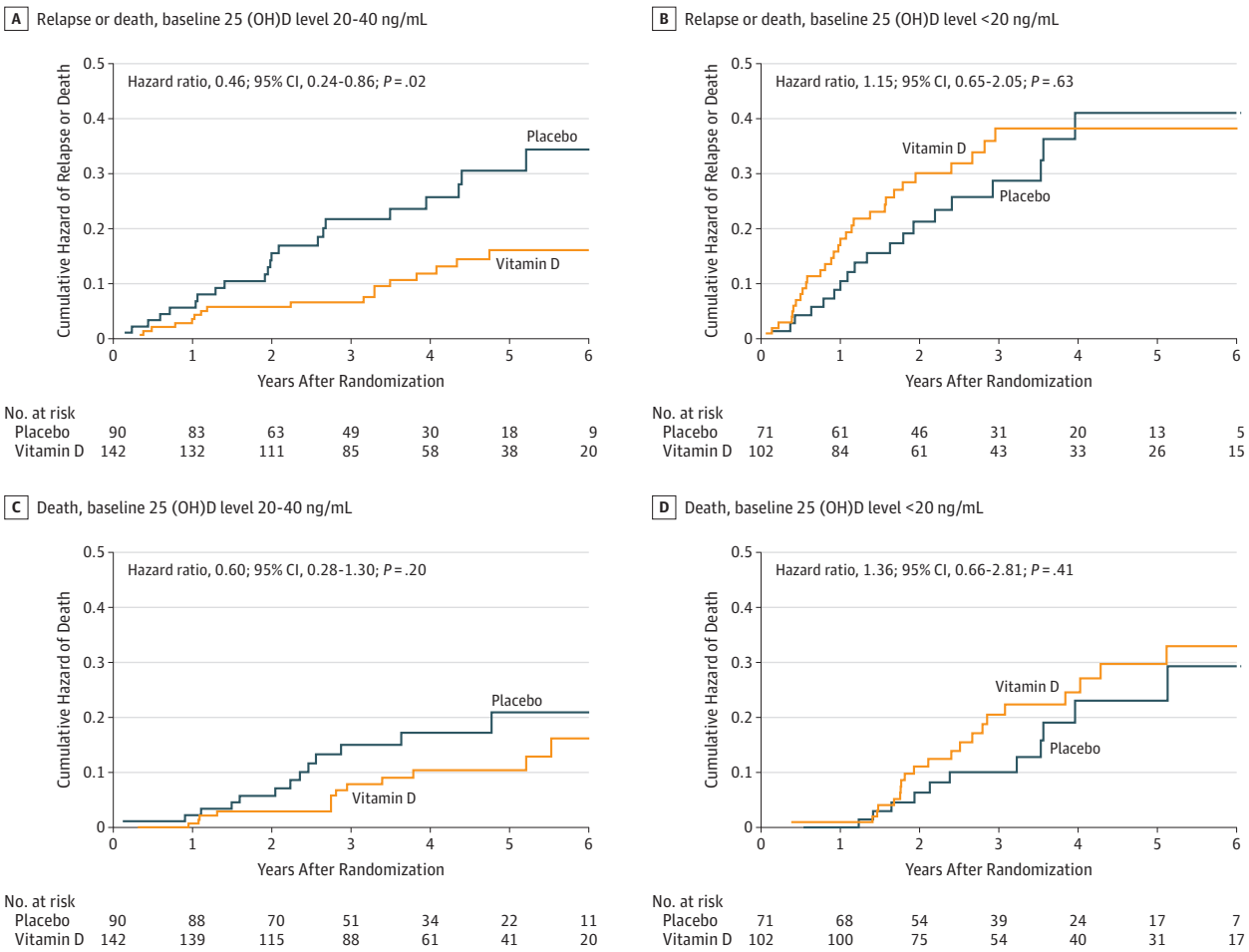
In the per-protocol analysis of participants continuing to take study medication until censoring, fractures occurred in 3 patients (1.3%) in the vitamin D group and 5 patients (3.4%) in the placebo group; urinary stones occurred in 2 (0.9%) vs 0 patients, respectively (**Table 3**). No patients developed hypercalcemia during the follow-up period.

Table 2. Effect of Vitamin D Supplementation on Relapse, Cancer-Specific Death, and Noncancer Death[a]

Outcomes	Total Study Population	Baseline Serum 25-Hydroxyvitamin D Level, ng/mL		*P* Value for Interaction
		<20	20-40	
Relapse				.04
Subdistribution HR (95% CI)	0.75 (0.48-1.17)	1.18 (0.64-2.19)	0.44 (0.21-0.89)	
P value	.21	.59	.02	
Cancer-specific death				.35
HR (95% CI)	1.09 (0.58-2.01)	1.45 (0.63-3.38)	0.78 (0.29-2.10)	
P value	.80	.38	.63	
Noncancer death				.27
HR (95% CI)	0.70 (0.29-1.73)	1.11 (0.26-4.65)	0.39 (0.11-1.39)	
P value	.44	.89	.15	

[a] Hazard ratio (HR) values greater than 1 indicate that vitamin D supplementation was associated with a decreased probability of the outcome.

Figure 3. Subgroup Analysis



Nelson-Aalen cumulative hazard curves are shown for relapse or death in the subgroups of (A) middle (20-40 ng/mL) and (B) low (<20 ng/mL) serum hydroxyvitamin D (25[OH]D) baseline levels and for total deaths in the subgroups of (C) middle and (D) low serum 25(OH)D baseline levels. There were only 5 patients with high (>40 ng/mL) 25(OH)D baseline levels; this group was not evaluated. Numbers at risk for panel C are not given because of weighting. Median observation times for relapse or death in the middle 25(OH)D subgroup were, for placebo, 3.4 (interquartile range [IQR], 1.9-4.5) years, and for vitamin D, 3.8 (IQR, 2.4-5.3) years; and in the low 25(OH)D subgroup, for placebo, 2.8 (IQR, 1.8-4.4) years, and for vitamin D, 2.5 (IQR, 1.6-5.2) years. Median observation times for total deaths in the middle 25(OH)D subgroup were, for placebo, 3.5 (IQR, 2.3-5.0) years, and for vitamin D, 3.8 (IQR, 2.5-5.4) years; and in the low 25(OH)D subgroup, for placebo, 3.4 (IQR, 2.1-5.0 years), and for vitamin D, 3.3 (IQR, 1.9-5.4) years.

Table 3. Safety Outcomes

Outcomes	No. (%) of Participants					
	Per Protocol[a]				By Randomization Group[b]	
	Adherent Until Censoring		Adherent for 1 y			
	Vitamin D (n = 227)	Placebo (n = 147)	Vitamin D (n = 243)	Placebo (n = 160)	Vitamin D (n = 251)	Placebo (n = 166)
Fracture	3 (1.3)	5 (3.4)	3 (1.2)	6 (3.8)	3 (1.2)	6 (3.6)
Urinary stones	2 (0.9)	0 (0.0)	4 (1.6)	2 (1.3)	4 (1.6)	4 (2.4)
Severe adverse events[c]	19 (8.4)	9 (6.1)	20 (8.2)	13 (8.1)	21 (8.4)	15 (9.0)
Cancer de novo[d]	15 (6.6)	8 (5.4)	16 (6.6)	9 (5.6)	16 (6.4)	9 (5.4)

[a] Safety outcomes were analyzed per protocol (ie, patients who continued to take study medication until censorship or for more than 1 year) because per-protocol analysis is considered to be more sensitive for safety outcomes. One participant lost to follow-up in the vitamin D group was considered nonadherent.

[b] Analyzed according to randomization group independent of adherence.

[c] Adverse events that resulted in admission.

[d] Cancer that appeared de novo in organs other than the site of the primary cancer after starting study medication.

Discussion

Among patients with digestive tract cancer, vitamin D supplementation, compared with placebo, did not reduce risks of relapse or death, death due to any cause, or relapse.

A similar RCT that included 155 patients found that 1200 IU/d of vitamin D supplementation did not improve relapse-free survival or overall survival of patients with non–small cell lung cancer.[23] However, a meta-analysis of 64 observational studies with a total of 44 165 patients with cancer found higher 25(OH)D levels to be associated with better progression-free and overall survival.[24] In addition, a meta-analysis of individual participant data from 8 cohort studies in Europe and the United States found higher cancer mortality in patients in the lowest quintiles of 25(OH)D levels among people with a history of cancer.[25] Discrepancies between the results of these RCTs and meta-analyses of observational studies suggest that higher 25(OH)D levels can be largely confounded by healthy lifestyles[26] that include daily physical activity.[27,28]

The recent VITAL study, an RCT that also used a vitamin D dosage of 2000 IU/d for primary prevention of cancer, enrolled 25 871 participants without a history of cancer. In that study, there was no reduction in risk of cancer mortality, but a post hoc analysis suggested a possible benefit of vitamin D after exclusion of early follow-up data.[29] A meta-analysis[30] of 3 other RCTs[8,31,32] found that vitamin D supplementation was significantly associated with lower total cancer mortality, even though each individual trial had null results. The study population in the current trial included distinct entities with biological and clinical differences—eg, esophageal cancer, gastric cancer, or colorectal cancer; stage I, II, or III cancer; and adenocarcinoma and nonadenocarcinoma. However, the power may have been too low to detect differences in these subgroups. It may therefore be useful to include the data in this trial in an individual patient data meta-analysis.

In the current study, vitamin D was effective only in a subgroup of patients with middle (20-40 ng/mL) serum 25(OH)D levels at baseline. However, this finding must be considered exploratory and interpreted with caution in the context of the null findings for the primary outcome measures in the total population, as well as the potential for type I error due to multiple comparisons. It was hypothesized that vitamin D would be effective in the subgroup with low 25(OH)D at baseline, as was observed in an RCT for a subgroup of patients with lung cancer (although with a nonsignificant interaction test).[24] It is possible that the optimal range of serum 25(OH)D levels with respect to survival may be quite different among types of cancers. In addition, the supplement dosage of 2000 IU/d in the trial may have been insufficient to increase vitamin D levels sufficiently in the subgroup with low 25(OH)D levels.

Findings regarding adverse events must also be considered exploratory because the study was not designed with sufficient power to detect significant differences. However, the relatively high dosage of vitamin D did not appear to be associated with frequent adverse events.

Limitations

This trial has several limitations. First, the study population included patients with a mixture of cancers with biological and clinical differences. Although post hoc subgroup analyses were performed for each tumor type and stage and for adenocarcinoma vs nonadenocarcinoma, the sample size may have been too small to detect significant differences in each subgroup. Second, patients were periodically examined (every 1-6 months) as outpatients by computed tomography, magnetic resonance imaging, positron emission tomography, and other procedures to exclude cancer relapse as deemed necessary. This variability in follow-up mode and timing may have led to inaccuracy in measured time to relapse. Third, approximately 10% of the participants stopped taking study medication during the trial, and adherence was based only on patient self-report, which may have caused a bias toward null results. Fourth, only 5 patients had high levels of 25(OH)D at baseline, too few to allow statistical evaluation. Fifth, the prespecified 25(OH)D cutoffs of 20 ng/mL and 40 ng/mL were based on published reports of primary prevention of incident cancer, but these cutoff points might be inappropriate in a clinical trial of secondary prevention of cancer relapse and death. Sixth, 7 patients had missing 25(OH)D levels, although multiple imputation produced consistent results. Seventh, radiological images were not reviewed centrally by a blinded independent third party, although the trial was double blinded.

Conclusions

Among patients with digestive tract cancer, vitamin D supplementation, compared with placebo, did not result in significant improvement in relapse-free survival at 5 years.

ARTICLE INFORMATION

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Author Contributions: Dr Urashima had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Concept and design: Urashima, Ohdaira, Yoshida, Kitajima, Suzuki.
Acquisition, analysis, or interpretation of data: Urashima, Akutsu, Okada, Yoshida.
Drafting of the manuscript: Urashima, Ohdaira, Yoshida, Kitajima.
Critical revision of the manuscript for important intellectual content: Akutsu, Okada, Suzuki.
Statistical analysis: Urashima, Yoshida.
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Administrative, technical, or material support: Ohdaira, Akutsu, Okada, Suzuki.
Supervision: Okada, Kitajima.

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REFERENCES

1. Bikle D. Nonclassic actions of vitamin D. *J Clin Endocrinol Metab*. 2009;94(1):26-34. doi:10.1210/jc.2008-1454

2. van Harten-Gerritsen AS, Balvers MGJ, Witkamp RF, Kampman E, van Duijnhoven FJB. Vitamin D, inflammation, and colorectal cancer progression: a review of mechanistic studies and future directions for epidemiological studies. *Cancer Epidemiol Biomarkers Prev*. 2015;24(12):1820-1828. doi:10.1158/1055-9965.EPI-15-0601

3. Garland CF, Comstock GW, Garland FC, Helsing KJ, Shaw EK, Gorham ED. Serum 25-hydroxyvitamin D and colon cancer: eight-year prospective study. *Lancet*. 1989;2(8673):1176-1178. doi:10.1016/S0140-6736(89)91789-3

4. Giovannucci E, Liu Y, Rimm EB, et al. Prospective study of predictors of vitamin D status and cancer incidence and mortality in men. *J Natl Cancer Inst*. 2006;98(7):451-459. doi:10.1093/jnci/djj101

5. Ng K, Meyerhardt JA, Wu K, et al. Circulating 25-hydroxyvitamin d levels and survival in patients with colorectal cancer. *J Clin Oncol*. 2008;26(18):2984-2991. doi:10.1200/JCO.2007.15.1027

6. Ng K, Wolpin BM, Meyerhardt JA, et al. Prospective study of predictors of vitamin D status and survival in patients with colorectal cancer. *Br J Cancer*. 2009;101(6):916-923. doi:10.1038/sj.bjc.6605262

7. Mezawa H, Sugiura T, Watanabe M, et al. Serum vitamin D levels and survival of patients with colorectal cancer: post-hoc analysis of a prospective cohort study. *BMC Cancer*. 2010;10(1):347. doi:10.1186/1471-2407-10-347

8. Wactawski-Wende J, Kotchen JM, Anderson GL, et al; Women's Health Initiative Investigators. Calcium plus vitamin D supplementation and the risk of colorectal cancer. *N Engl J Med*. 2006;354(7):684-696. doi:10.1056/NEJMoa055222

9. Lappe JM, Travers-Gustafson D, Davies KM, Recker RR, Heaney RP. Vitamin D and calcium supplementation reduces cancer risk: results of a randomized trial. *Am J Clin Nutr*. 2007;85(6):1586-1591. doi:10.1093/ajcn/85.6.1586

10. Heist RS, Zhou W, Wang Z, et al. Circulating 25-hydroxyvitamin D, VDR polymorphisms, and survival in advanced non-small-cell lung cancer. *J Clin Oncol*. 2008;26(34):5596-5602. doi:10.1200/JCO.2008.18.0406

11. Tamez S, Norizoe C, Ochiai K, et al. Vitamin D receptor polymorphisms and prognosis of patients with epithelial ovarian cancer. *Br J Cancer*. 2009;101(12):1957-1960. doi:10.1038/sj.bjc.6605414

12. Hama T, Norizoe C, Suga H, et al. Prognostic significance of vitamin D receptor polymorphisms in head and neck squamous cell carcinoma. *PLoS One*. 2011;6(12):e29634. doi:10.1371/journal.pone.0029634

13. Janssens W, Bouillon R, Claes B, et al. Vitamin D deficiency is highly prevalent in COPD and correlates with variants in the vitamin D-binding gene. *Thorax*. 2010;65(3):215-220. doi:10.1136/thx.2009.120659

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(2):634-640. doi:10.3945/ajcn.2008.26445

15. Japanese Gastric Cancer Association. Japanese gastric cancer treatment guidelines 2010 (ver 3). *Gastric Cancer*. 2011;14(2):113-123. doi:10.1007/s10120-011-0042-4

16. Watanabe T, Itabashi M, Shimada Y, et al; Japanese Society for Cancer of the Colon and Rectum. Japanese Society for Cancer of the Colon and Rectum (JSCCR) guidelines 2010 for the treatment of colorectal cancer. *Int J Clin Oncol*. 2012;17(1):1-29. doi:10.1007/s10147-011-0315-2

17. 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(3):529-533.

18. Uitterlinden AG, Fang Y, Van Meurs JB, Pols HA, Van Leeuwen JP. Genetics and biology of vitamin D receptor polymorphisms. *Gene*. 2004;338(2):143-156. doi:10.1016/j.gene.2004.05.014

19. Suzuki M, Yoshioka M, Hashimoto M, et al. 25-hydroxyvitamin D, vitamin D receptor gene polymorphisms, and severity of Parkinson's disease. *Mov Disord*. 2012;27(2):264-271. doi:10.1002/mds.24016

20. Schulz KF, Grimes DA. Multiplicity in randomised trials II: subgroup and interim analyses. *Lancet*. 2005;365(9471):1657-1661. doi:10.1016/S0140-6736(05)66516-6

21. Hess KR. Graphical methods for assessing violations of the proportional hazards assumption in Cox regression. *Stat Med*. 1995;14(15):1707-1723. doi:10.1002/sim.4780141510

22. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. *J Am Stat Assoc*. 1999;94(446):496-509. doi:10.1080/01621459.1999.10474144

23. Akiba T, Morikawa T, Odaka M, et al. Vitamin D supplementation and survival of patients with non-small cell lung cancer: a randomized, double-blind, placebo-controlled trial. *Clin Cancer Res*. 2018;24(17):4089-4097. doi:10.1158/1078-0432.CCR-18-0483

24. Vaughan-Shaw PG, O'Sullivan F, Farrington SM, et al. The impact of vitamin D pathway genetic variation and circulating 25-hydroxyvitamin D on cancer outcome: systematic review and meta-analysis. *Br J Cancer*. 2017;116(8):1092-1110. doi:10.1038/bjc.2017.44

25. Schöttker B, Jorde R, Peasey A, et al; Consortium on Health and Ageing: Network of Cohorts in Europe and the United States. Vitamin D and mortality: meta-analysis of individual participant data from a large consortium of cohort studies from Europe and the United States. *BMJ*. 2014;348:g3656. doi:10.1136/bmj.g3656

26. Van Blarigan EL, Fuchs CS, Niedzwiecki D, et al. Association of survival with adherence to the American Cancer Society nutrition and physical activity guidelines for cancer survivors after colon cancer diagnosis: the CALGB 89803/Alliance Trial. *JAMA Oncol*. 2018;4(6):783-790. doi:10.1001/jamaoncol.2018.0126

27. Skender S, Böhm J, Schrotz-King P, et al. Plasma 25-hydroxyvitamin D_3 levels in colorectal cancer patients and associations with physical activity. *Nutr Cancer*. 2017;69(2):229-237. doi:10.1080/01635581.2017.1265131

28. Morales-Oyarvide V, Meyerhardt JA, Ng K. Vitamin D and physical activity in patients with colorectal cancer: epidemiological evidence and therapeutic implications. *Cancer J*. 2016;22(3):223-231. doi:10.1097/PPO.0000000000000197

29. Manson JE, Cook NR, Lee I-M, et al; VITAL Research Group. Vitamin D supplements and prevention of cancer and cardiovascular disease. *N Engl J Med*. 2019;380(1):33-44. doi:10.1056/NEJMoa1809944

30. Keum N, Giovannucci E. Vitamin D supplements and cancer incidence and mortality: a meta-analysis. *Br J Cancer*. 2014;111(5):976-980. doi:10.1038/bjc.2014.294

31. Avenell A, MacLennan GS, Jenkinson DJ, et al; RECORD Trial Group. Long-term follow-up for mortality and cancer in a randomized placebo-controlled trial of vitamin D(3) and/or calcium (RECORD trial). *J Clin Endocrinol Metab*. 2012;97(2):614-622. doi:10.1210/jc.2011-1309

32. Trivedi DP, Doll R, Khaw KT. Effect of four monthly oral vitamin D_3 (cholecalciferol) supplementation on fractures and mortality in men and women living in the community: randomised double blind controlled trial. *BMJ*. 2003;326(7387):469. doi:10.1136/bmj.326.7387.469