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Marine ω-3 Polyunsaturated Fatty Acid and Fish Intake after Colon Cancer Diagnosis and Survival: CALGB 89803 (Alliance)

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No potential conflicts of interest were disclosed.

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Abstract

Background—Marine ω-3 polyunsaturated fatty acids (PUFAs), primarily found in dark fish, may prevent colorectal cancer progression, in part through inhibition of prostaglandin-endoperoxide synthase 2 (PTGS2). However, data in humans are limited.

Methods—We examined marine ω-3 PUFAs and fish intake and survival among 1,011 colon cancer patients enrolled in Cancer and Leukemia Group B 89803 between 1999 and 2001 and followed through 2009. Diet was assessed during and 6 months after chemotherapy. We used Cox proportional hazards regression to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) for disease-free (DFS), recurrence-free (RFS), and overall survival (OS).

Results—We observed 343 recurrences and 305 deaths (median follow-up: 7 years). Patients in the highest vs. lowest quartile of marine ω-3 PUFA intake had an HR for DFS of 0.72 (95% CI, 0.54–0.97; P_{trend} = 0.03). Individuals who consumed dark fish ≥1/week versus never had longer DFS (HR 0.65; 95% CI, 0.48–0.87; *P*-value =0.007), RFS (HR 0.61; 95% CI, 0.46–0.86; P_{trend} = 0.007), and OS (HR 0.68; 95% CI, 0.48–0.96; P_{trend} = 0.04). In a subset of 510 patients, the association between marine ω-3 PUFA intake and DFS appeared stronger in patients with high PTGS2 expression (HR 0.32; 95% CI, 0.11–0.95; P_{trend} = 0.01) compared with patients with absent/low PTGS2 expression (HR 0.78; 95% CI, 0.48–1.27; P_{trend} = 0.35; $P_{interaction}$ = 0.19).

Conclusions—Patients with high intake of marine ω-3 PUFAs and dark fish after colon cancer diagnosis may have longer DFS.

Impact—Randomized controlled trials examining dark fish and/or marine ω-3 PUFA supplements and colon cancer recurrence/survival are needed.

Introduction

More than 1.3 million Americans currently live with colorectal cancer (1). Although colorectal cancer remains the second-leading cause of cancer death in the United States, individuals with localized disease (40% of cases) have a 5-year survival of 90%, and individuals with regional disease (36% of cases) have a 5-year survival of 70% (2). The

lengthening survival of colorectal cancer patients presents an opportunity to intervene on lifestyle factors, such as diet, to improve clinical outcomes.

Preclinical data suggest that marine ω-3 polyunsaturated fatty acids (PUFA) inhibit the progression of colorectal cancer. *In vitro* studies have shown that marine ω-3 PUFAs reduce proliferation and invasion, increase apoptosis, and cell-cycle arrest, and may improve chemotherapy efficacy in human colorectal cancer cells (3, 4). In animal models, marine ω-3 PUFAs reduce tumor growth, inhibit metastasis, and increase survival (3, 5, 6). In addition, ω-3 PUFA supplementation in colorectal cancer patients may improve inflammatory markers, immune response, and physical function (7, 8). Several mechanisms have been identified that support an inhibitory effect of marine ω-3 PUFAs on colorectal cancer, including inhibition of prostaglandin-endoperoxide synthase 2 (PTGS2; also known as cyclooxygenase-2) expression and activity (3). However, studies in humans with clinical outcomes are limited (9).

We recently reported that stage I to III colorectal cancer survivors who increased their marine ω-3 PUFA intake after diagnosis by ≥0.15 g/d had a 70% lower risk of colorectal cancer mortality compared to those with little or no change in intake [hazard ratio (HR) 0.30; 95% confidence interval (CI), 0.14–0.64; ref. 10]. However, the post-diagnostic dietary assessment took place a median of 3 years after diagnosis, after the greatest risk of colorectal cancer recurrence has passed (11). In addition, we observed few events of colorectal cancer mortality ($n = 169$) and did not have data on tumor biomarkers. Thus, further studies are needed to evaluate whether marine ω-3 PUFAs are associated with disease-free survival in colorectal cancer, and to elucidate biological mechanisms.

In this study, we examined marine ω-3 PUFA intake in relation to disease-free survival among stage III colon cancer patients. We also examined whether tumor PTGS2 expression modified the association between marine ω-3 PUFAs and disease-free survival. Finally, to inform dietary recommendations for cancer survivors, we examined post-diagnostic fish intake in relation to disease-free survival. We hypothesized that colon cancer patients with higher intake of marine ω-3 PUFAs and dark fish, the primary source of these fats, would have longer disease-free survival, and that this association would be stronger among patients whose tumors overexpressed PTGS2.

Materials and Methods

Study population

Cancer and Leukemia Group B (CALGB) 89803, an adjuvant chemotherapy trial, enrolled 1,264 colon cancer patients between 1999 and 2001. CALGB is now part of the Alliance for Clinical Trials in Oncology. Eligibility criteria and the results of the main trial have been reported (11). Briefly, patients had completely resected stage III adenocarcinoma of the colon with no metastases, no prior chemotherapy or radiotherapy, performance scale 0 to 2, and adequate bone marrow, liver, and kidney function. This study was conducted in accordance with recognized ethical guidelines and approved by each site's Institutional Review Board; all participants signed an informed consent. Participants were asked to complete a questionnaire at baseline during chemotherapy and 6 months post-chemotherapy;

1,095 participants (87%) completed the baseline questionnaire and 981 participants completed the second questionnaire (78%). We excluded eight individuals whose cancer recurred prior to the baseline questionnaire. To reduce potential bias due to reverse causation, we also excluded 30 individuals who recurred or died within 90 days of the baseline questionnaire. Finally, we excluded 46 patients who reported implausible energy intake (<600 or >4,200 for men, <500 or >3,200 for women) or left ≥70 items on the baseline questionnaire blank (12), leaving 1,011 patients eligible for analysis.

Dietary assessment

Diet was assessed twice using a validated semiquantitative food frequency questionnaire (FFQ), at baseline during chemotherapy (approximately 3 months after diagnosis) and 6 months after chemotherapy (approximately 15 months after diagnosis). The FFQ assessed intake of 131 foods and beverages over the past 3 months in up to nine frequency categories ranging from never to 6+per day (13, 14). The duration and dosage of supplements was also assessed. Nutrient data were obtained from the USDA. For our main analysis, dietary data from the two FFQs were weighted proportional to follow-up to reduce measurement error and for consistency with prior publications (12, 15). Data from the first FFQ was used to classify exposure between FFQ 1 and FFQ 2 and the average intake between FFQ 1 and FFQ 2 was used to classify exposure after FFQ 2.

Marine ω-3 PUFA intake (g/day) was calculated by multiplying the amount in the specified portion of each item on the FFQ (including supplements) by the frequency of its use and summing across items. Five questions were used to assess fish intake: canned tuna fish (3–4 oz.); breaded fish cakes, pieces, or fish sticks (1 serving, store bought); shrimp, lobster, scallops, clams as a main dish (1 serving); dark meat fish, for example, mackerel, salmon, sardines, bluefish, swordfish (3–5 oz.); and other fish, for example, cod, haddock, halibut (3–5 oz.). Fish oil (omega-3 fatty acids) use was queried with the following response options: never taken; taken in the past only; or yes, currently take it. Only 24 (2%) patients reported taking fish oil.

The FFQ has been validated, including among cancer patients on chemotherapy (13). The correlation coefficient for dark fish intake assessed by the FFQ and diet records was 0.58 (16), and eicosapentaenoic acid intake from the FFQ was correlated with levels in subcutaneous fat aspirates ($r = 0.47$; ref. 17).

Tumor PTGS2 expression

Tumor PTGS2 expression data were available for 510 patients. Baseline characteristics and survival outcomes of patients with tumor blocks available were comparable to those without tissue data (18). PTGS2 immunostaining was performed by Dr. Shuji Ogino's laboratory (not CLIA-certified) at the Dana-Farber Cancer Institute using published methods (19). Cancer tissue with PTGS2 overexpression (positive control) and normal colonic tissue (negative control) were included in each assay run. Dr. Ogino evaluated PTGS2 expression using a standardized scoring system (absent, weak, moderate, strong). A second pathologist reviewed a random sample of 108 tissue sections; the concordance was 0.92 ($\kappa = 0.62$; $P <$

0.001). Pathologists were blinded to patient-level data. Tumors with moderate or strong immunostaining were classified as high PTGS2 expression (19).

Outcome ascertainment and follow-up

Our primary outcome was disease-free survival, defined as time from the baseline FFQ to tumor recurrence, occurrence of a new primary colon tumor, or death from any cause, whichever came first. We also assessed recurrence-free and overall survival. Recurrence-free survival was defined as time from the baseline FFQ to tumor recurrence or occurrence of a new primary colon tumor; patients who died without known recurrence were censored at last evaluation. Overall survival was defined as time from the baseline FFQ to death from any cause. Patients in CALGB 89803 were followed for a minimum of 7 years after treatment; median follow-up was 7 years and nearly 100% complete.

Statistical analysis

There was no difference in survival between treatment arms in CALGB 89803 (11). Therefore, we combined data from all 1,011 eligible participants and compared disease-free, recurrence-free, and overall survival by post-diagnostic marine ω-3 PUFA and fish intake.

We used the nutrient residual method to adjust marine ω-3 PUFAs for energy intake and categorized the adjusted levels into quartiles (12). We also dichotomized marine ω-3 PUFA intake at 0.25 g/d, the level of intake consistent with the 2015 US Dietary Guidelines (20). We categorized fish items based on the intake in the study population: never, <1/month, 1–3/month, and ≥1/week for dark fish; other fish; breaded fish; and shrimp, lobsters, scallops, clams as a main dish and <1/month, 1–3/month, 1/week, and >1/ week for canned tuna.

We used the Kaplan–Meier method and log-rank test to compare disease-free survival by marine ω-3 PUFA intake after diagnosis (21). We next used Cox proportional hazards regression to examine marine ω-3 PUFA and fish intake in relation to disease-free, recurrence-free, and overall survival (22). Model 1 was adjusted for sex, energy intake (kcal/d), and age (years). Model 2 was additionally adjusted for T-stage (T1–T2, T3–T4, unknown), number of positive lymph nodes (1–3, ≥4, unknown), baseline performance status (0, 1–2, unknown), treatment arm (irinotecan: yes, no), body mass index (BMI; kg/m^2), physical activity (metabolic equivalent task-hours per week; MET-h/week), smoking (current, past, never, unknown), and aspirin use (yes, no, unknown). We used the missing indicator method to adjust for categorical variables with missing data. Eleven patients (1%) were missing BMI or physical activity; these patients were excluded from analyses adjusted for these factors. We considered adjustment for median household income, western dietary pattern, prudent dietary pattern, saturated fat, mono-unsaturated fat, ω-6 PUFA, *trans* fat, protein, alcohol, carbohydrate, glycemic load, folate, vitamin D, and calcium, but the point estimates did not materially change and we omitted these variables. We confirmed that the proportional hazards assumption was valid by including the cross-product of marine ω-3 PUFAs and time in our model ($P = 0.64$).

We hypothesized that the association between marine ω-3 PUFAs and disease-free survival would be stronger among patients with tumors that overexpress PTGS2. Therefore, we performed a stratified analysis (tumor PTGS2 absent/low vs. high) among the 510 patients

with biomarker data (19). We used a Wald test to calculate a $P_{interaction}$ for the cross-product of marine ω-3 PUFA intake and PTGS2 expression status in Model 2.

We conducted several sensitivity analyses. We explored whether the association between marine ω-3 PUFAs and disease-free survival was modified by age (<60, ≥60 years), sex, treatment (irinotecan: yes, no), BMI (<30, ≥30 kg/m^2), physical activity (<18, ≥18 MET-h/wk), or aspirin use (yes, no). To do so, we created cross-product terms between marine ω-3 PUFA intake and dichotomized effect modifiers and used Wald tests to evaluate whether there was evidence of interaction. Next, individuals undergoing chemotherapy may change their diet as a result of their treatment. Therefore, we examined the difference in marine ω-3 PUFA intake reported on the first and second questionnaire, and assessed marine ω-3 PUFA intake reported on the second FFQ in relation to disease-free survival. The second FFQ was administered 6 months after completion of chemotherapy, when patients are likely consuming their usual post-diagnostic diet. For this analysis, follow-up started at the second FFQ. Finally, to further assess potential bias due to reverse causation, we additionally excluded individuals who recurred or died 90 to 180 days after the baseline questionnaire (individuals who recurred or died within 90 days of the baseline questionnaire were excluded in our primary analyses).

Data collection was performed by the Alliance Statistics and Data Center. Analyses were conducted using SAS v. 9.4 and two-sided *P*-values <0.05 were considered statistically significant. All analyses were based on the study database frozen on November 9, 2009.

Results

Characteristics of the 1,011 colon cancer patients are presented in Table 1. Patients with the highest intake of marine ω-3 PUFAs were less likely to be white or current smokers, more likely to take fish oil, and performed more physical activity compared to patients with the lowest intake. There were no differences in sex, age, performance status, stage, bowel abnormalities, grade, positive lymph nodes, treatment, aspirin, or BMI.

Higher intake of marine ω-3 PUFAs after colon cancer diagnosis was associated with longer disease-free survival. Compared to patients in the lowest quartile, patients in the highest quartile had an HR for disease-free survival of 0.72 (95% CI, 0.54–0.97; P_{trend} = 0.03; Fig. 1A; Table 2). When dichotomizing marine ω-3 PUFA intake at 0.25 g/d (the level consistent with the 2015 US Dietary Guidelines), patients consuming ≥0.25 g/d had an adjusted HR for disease-free survival of 0.80 (95% CI, 0.64–1.00; *P*-value = 0.05; Fig. 1B). This association appeared to be driven by cancer outcomes; patients in the highest quartile of marine ω-3 PUFA intake had a HR for recurrence-free survival of 0.70 (95% CI, 0.51–0.96; P_{trend} = 0.02). Post-diagnostic marine ω-3 PUFA intake was inversely, but not statistically significantly, associated with overall survival (HR highest vs. lowest quartile: 0.81; 95% CI, 0.58–1.15; P_{trend} = 0.23).

The association between marine ω-3 PUFA intake and disease-free survival appeared to be modified by tumor PTGS2 expression, although the test for interaction was not statistically significant ($P_{interaction}$ = 0.19; Table 3). Among the 163 patients with PTGS2-high tumors,

patients in the highest versus lowest quartile of post-diagnostic marine ω-3 PUFA intake had an adjusted HR for disease-free survival of 0.32 (95% CI, 0.11–0.95; P_{trend} = 0.01). There was no statistically significant association among the 347 patients with PTGS2-absent/low tumors (HR 0.78; 95% CI, 0.48–1.27; P_{trend} = 0.35).

The association between marine ω-3 PUFA intake and disease-free survival did not differ by age ($P_{interaction}$ = 0.82), sex ($P_{interaction}$ = 0.84), performance status ($P_{interaction}$ = 0.60), treatment ($P_{interaction}$ = 0.90), BMI ($P_{interaction}$ = 0.99), physical activity ($P_{interaction}$ = 0.35), or aspirin ($P_{interaction}$ = 0.35). In addition, the majority of patients did not change their intake of marine ω-3 PUFAs between the first and second questionnaire. Point estimates did not meaningfully change when we categorized patients based on the second questionnaire only (highest vs. lowest quartile; HR 0.79; 95% CI, 0.53–1.16) or when excluding individuals who experienced an event within 180 days of the baseline questionnaire (highest vs. lowest quartile; HR 0.74; 95% CI, 0.54–1.01).

Consistent with the marine ω-3 PUFA findings, colon cancer patients who consumed dark fish ≥1/week compared to never had an HR for disease-free survival of 0.65 (95% CI, 0.48–0.87; *P*-value: 0.007; Table 4). Dark fish was also associated with longer recurrence-free (HR for ≥1/week vs. never: 0.63; 95% CI, 0.46–0.86; *P*-value = 0.007) and overall (HR ≥ 1/week vs. never: 0.68; 95% CI, 0.48–0.96; *P*-value = 0.04) survival. No other fish or seafood was associated with disease-free, recurrence-free, or overall survival.

Discussion

In this prospective analysis of 1,011 colon cancer patients, individuals with high intake of marine ω-3 PUFAs had longer disease-free survival. We also observed that patients who consumed dark fish ≥1/week had a 35% lower risk of cancer recurrence or death compared with those who consumed none.

Our group recently examined marine ω-3 PUFA intake after colorectal cancer diagnosis in relation to mortality (10). In that study, individuals who consumed ≥0.3 g/d of marine ω-3 PUFAs had a nonstatistically significant 41% lower risk of colorectal cancer mortality compared with individuals consuming <0.1 g/d (95% CI, 0.35–1.01; P_{trend} = 0.03). Further, patients who increased their marine ω-3 PUFA intake by ≥0.15 g/d compared to before diagnosis had a 70% lower risk of mortality compared with those who made little or no change (95% CI, 0.14–0.64; P_{trend} < 0.001). The results of our current study complement and expand upon the prior report. Patients in the previous study had to survive at least 2 years after diagnosis to complete the post-diagnostic dietary questionnaire (the median time from diagnosis to the questionnaire approached 3 years; ref. 11). The participants in our current analysis completed a dietary questionnaire an average of 3 months after diagnosis and approximately 1 year later.

In addition, our current study had tumor PTGS2 expression data in a subset of participants. Although the test for interaction was not statistically significant, marine ω-3 PUFA intake appeared associated with improved disease-free survival among individuals with high PTGS2 expression but not those with low PTGS2 expression. PTGS2 enzymes convert

arachidonic acid into prostaglandins, which promote inflammation and tumor growth. PTGS2 is overexpressed in many colon tumors (32% in our cohort), and individuals with higher PTGS2 expression have worse survival (23). *In vitro* studies have shown that marine ω-3 PUFAs inhibit PTGS2 expression in colon cancer cells (3). Although our results should be interpreted cautiously given our small sample size for evaluating effect modification, these data support the hypothesis that marine ω-3 PUFAs reduce risk of colon cancer recurrence and death in part through inhibition of PTGS2 and inflammation.

Further, we examined dietary sources of marine ω-3 PUFAs in relation to disease-free survival. Endogenous synthesis of long-chain ω-3 PUFAs is limited; the majority of these fatty acids are obtained from fish or fish oil. Dark fish is particularly high in marine ω-3 PUFAs. For example, 100 g of salmon (a “dark fish”) contains approximately 2 g of marine ω-3 PUFAs, whereas 100 g of cod (an “other fish”) contains <0.2 g (24). Based on unpublished data from the Health Professionals Follow-up Study, dark fish provided approximately 50% of the marine ω-3 PUFAs consumed in the United States at the time that our study was initiated (1999–2001). Thus, although data from randomized controlled trials are needed, our study supports the recommendation that colon cancer patients follow the 2015 U.S. Dietary Guidelines to consume eight or more ounces per week of seafood, and seafood choices with higher amounts of marine ω-3 PUFAs (i.e., dark/fatty fish) should be included (20).

Strengths of our study include prospective, repeated assessments of diet using a validated FFQ; large number of cancer recurrences and deaths; standardized cancer treatment and surveillance; high follow-up rate; and tumor PTGS2 expression data. Our study also has limitations. First, participants in a clinical trial may not be representative of all colon cancer patients. Second, although we adjusted for known risk factors for colon cancer recurrence and survival, we cannot exclude the possibility of confounding. Nonetheless, the consistency of our findings for the nutrient, its main food source, and evidence of an interaction with a tumor biomarker in an *a priori* identified biologic mechanism are supportive of a true association. Third, it is possible that the association that we observed reflects intake prior to diagnosis. However, the association between post-diagnostic marine ω-3 PUFAs and colorectal cancer mortality was independent of pre-diagnostic intake in the Health Professionals Follow-up Study and Nurses’ Health Study cohorts (10). Finally, we were unable to examine fish oil use in relation to disease-free survival, or separate the effects of marine ω-3 PUFAs from food versus supplements, due to low use of fish oil in our study population. Thus, the question remains whether fish intake is required or if a supplemental dose could achieve the same benefit that we observed in this study.

In conclusion, colon cancer patients who consumed higher amounts of marine ω-3 PUFAs and dark fish after diagnosis had longer disease-free survival. Patients with high PTGS2 expression in their tumors had the greatest reduction in risk of cancer recurrence and death. Randomized controlled trials examining dark fish and/or marine ω-3 PUFA supplements after colon cancer diagnosis in relation to cancer recurrence are needed.

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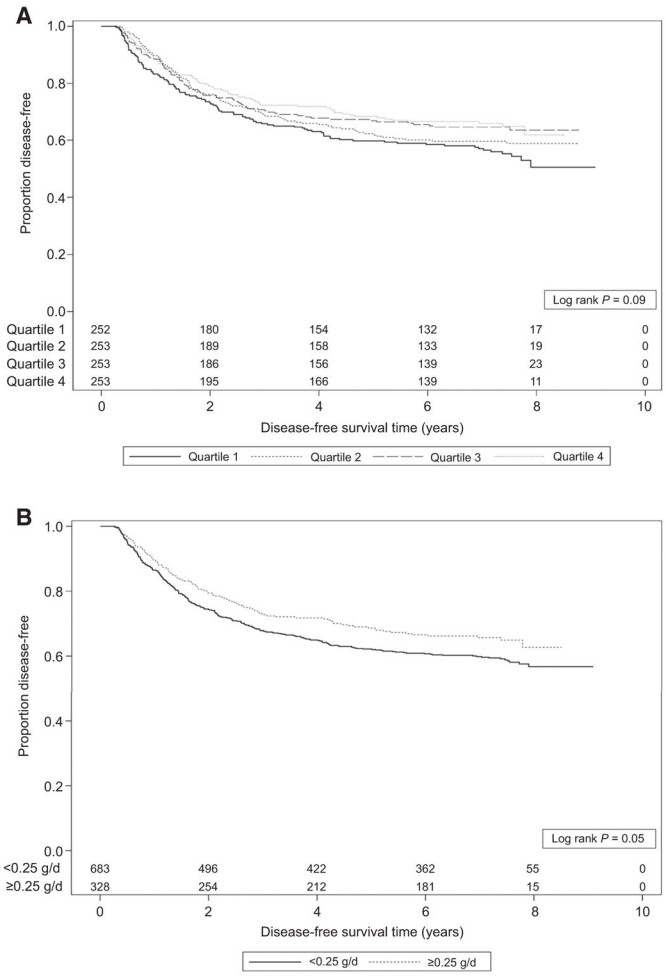

Figure 1.

A, Disease-free survival among 1,011 stage III colon cancer patients, by post-diagnostic marine ω-3 PUFA intake. **B,** Disease-free survival among 1,011 stage III colon cancer patients, by post-diagnostic marine ω-3 PUFA intake dichotomized at the 2015 U.S. Dietary Guidelines recommended intake level of 250 mg/day.

Table 1

Characteristics of 1,011 stage III colon cancer patients by post-diagnostic intake of marine ω-3 PUFAs

	Quartile of marine ω-3 PUFA intake				
Characteristic, median [interquartile range] or *N* (%)	**1**	**2**	**3**	**4**	***P*-value**
N	252	253	253	253	
Median marine ω-3 PUFA intake, g/d	0.09 [0.07, 0.11]	0.15 [0.14, 0.17]	0.23 [0.21, 0.25]	0.40 [0.33, 0.57]	
Male	156 (62)	137 (54)	144 (57)	132 (52)	0.14
Age, y	60 [50, 69]	60 [51, 68]	61 [53, 70]	60 [52, 69]	0.21
Race					0.007
White	235 (93)	229 (91)	224 (89)	211 (83)	
Black	8 (3)	16 (6)	20 (8)	21 (8)	
Other	9 (4)	8 (3)	9 (4)	21 (8)	
Baseline performance status					0.25
Fully active	184 (73)	193 (76)	188 (74)	177 (70)	
Ambulatory but restricted in strenuous activity	65 (26)	57 (23)	60 (24)	66 (26)	
Unknown	3 (1)	3 (1)	5 (2)	10 (4)	
Invasion through bowel wall					0.44
T1–T2	33 (13)	31 (12)	30 (12)	42 (17)	
T3–T4	202 (80)	210 (83)	204 (81)	191 (75)	
Unknown	17 (7)	12 (5)	19 (8)	20 (8)	
Positive lymph nodes					0.53
1–3 (N1)	154 (61)	161 (64)	154 (61)	166 (66)	
≥4 (N2)	94 (37)	89 (35)	94 (37)	79 (31)	
Unknown	4 (2)	3 (1)	5 (2)	8 (3)	
Clinical bowel abnormality					
Perforation	10 (4)	13 (5)	13 (5)	7 (3)	0.70
Obstruction	52 (21)	64 (25)	50 (20)	56 (22)	0.37
Grade of differentiation					0.39
Well	11 (4)	18 (7)	10 (4)	18 (7)	
Moderate	177 (70)	171 (68)	176 (70)	177 (70)	
Poor	61 (24)	61 (24)	60 (24)	50 (20)	

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	Quartile of marine ω-3 PUFA intake				
Characteristic, median [interquartile range] or *N* (%)	**1**	**2**	**3**	**4**	***P*-value**
Unknown	3 (1)	3 (1)	7 (3)	8 (3)	
Treatment arm					0.97
Fluorouracil + leucovorin	129 (51)	125 (49)	129 (51)	130 (51)	
Irinotecan, fluorouracil, leucovorin	123 (49)	128 (51)	124 (49)	123 (49)	
Smoking status					<0.001
Current	32 (13)	32 (13)	17 (7)	22 (9)	
Past	109 (43)	113 (45)	98 (39)	126 (50)	
Never	111 (44)	100 (40)	136 (54)	104 (41)	
Unknown	0 (0)	8 (3)	2 (1)	1 (0)	
Aspirin user	22 (9)	21 (8)	17 (7)	21 (8)	0.52
Fish oil supplement user	0 (0)	0 (0)	0 (0)	24 (9)	<0.001
Energy intake, kcal/d	1854 [1454, 2334]	1860 [1521, 2294]	2001 [1542, 2460]	1850 [1505, 2240]	0.05
BMI, kg/m^2	27.9 [24.5, 31.9]	27.9 [24.0, 32.4]	27.6 [24.4, 31.4]	27.4 [24.5, 31.5]	0.80
Physical activity, MET h/wk	5.3 [1.5, 16.9]	6.1 [2.0, 17.3]	7.7 [2.6, 17.0]	9.0 [3.5, 22.0]	0.004

Table 2

Post-diagnostic marine ω-3 PUFA intake in relation to cancer recurrence and survival among 1,011 stage III colon cancer patients

	Quartile of marine ω-3 PUFAs				
	1	**2**	**3**	**4**	P_{trend}[a]
No. at risk	252	253	253	253	
Disease-free survival[b]					
Events	112	101	88	85	
Model 1 HR (95% CI)[c]	1.00	0.88 (0.67–1.15)	0.77 (0.58–1.02)	0.73 (0.55–0.97)	0.03
Model 2 HR (95% CI)[d]	1.00	0.84 (0.64–1.10)	0.74 (0.56–0.98)	0.72 (0.54–0.97)	0.03
Recurrence-free survival[b]					
Events	96	95	81	71	
Model 1 HR (95% CI)[c]	1.00	0.95 (0.72–1.26)	0.83 (0.62–1.11)	0.71 (0.52–0.96)	0.02
Model 2 HR (95% CI)[d]	1.00	0.91 (0.68–1.21)	0.78 (0.58–1.05)	0.70 (0.51–0.96)	0.02
Overall survival					
Events	88	81	68	68	
Model 1 HR (95% CI)[c]	1.00	0.91 (0.66–1.26)	0.69 (0.49–0.98)	0.79 (0.56–1.10)	0.13
Model 2 HR (95% CI)[d]	1.00	0.86 (0.62–1.19)	0.68 (0.48–0.96)	0.81 (0.58–1.15)	0.23

[a] *P*-trend calculated by modeling the median of each category as a continuous term.

[b] Disease-free survival = time from the baseline FFQ to tumor recurrence, occurrence of a new primary colon tumor, or death from any cause, whichever came first. Recurrence-free survival =time from the baseline FFQ to tumor recurrence or occurrence of a new primary colon tumor; patients who died without known recurrence were censored at last evaluation.

[c] Cox proportional hazards regression model adjusted for age, sex, and energy intake.

[d] Cox proportional hazards regression model adjusted for variables in Model 1 plus T-stage, number of positive lymph nodes, baseline performance status, treatment arm, body mass index, physical activity, smoking, and aspirin use.

Table 3

Post-diagnostic marine ω-3 PUFA intake in relation to cancer recurrence or death among 510 stage III colon cancer patients by tumor PTGS2 expression status ($P_{interaction}$ = 0.19)

	Quartile of marine ω-3 PUFAs				
Tumor PTGS2 expression	**1**	**2**	**3**	**4**	P_{trend}[a]
Absent/low					
Events/no. at risk	44/97	32/84	29/84	29/82	
HR (95% CI)[b]	1.00	0.86 (0.53–1.38)	0.83 (0.52–1.35)	0.78 (0.48–1.27)	0.35
High					
Events/no. at risk	13/38	24/56	11/35	5/34	
HR (95% CI)[b]	1.00	1.07 (0.52–2.22)	0.67 (0.27–1.65)	0.32 (0.11–0.95)	0.01

[a] P_{trend} calculated by modeling the median of each category as a continuous term.

[b] Cox proportional hazards regression model adjusted for age, sex, and energy intake, T-stage, number of positive lymph nodes, baseline performance status, treatment arm, body mass index, physical activity, smoking, and aspirin use.

Table 4

Post-diagnostic fish and seafood intake after diagnosis in relation to cancer recurrence and mortality among 1,011 stage III colon cancer patients

	Dark meat fish (3–5 oz.)[a]				
	Never	**<1/month**	**1–3/month**	**≥1/week**	P_{trend}[b]
Disease-free survival[c]					
Events	134	62	115	75	
Model 1 HR (95% CI)[d]	1.0	0.77 (0.57–1.04)	0.77 (0.60–0.99)	0.63 (0.47–0.84)	0.003
Model 2 HR (95% CI)[e]	1.0	0.74 (0.55–1.00)	0.79 (0.61–1.02)	0.65 (0.48–0.87)	0.007
Overall survival					
Events	109	46	90	60	
Model 1 HR (95% CI)[d]	1.0	0.81 (0.57–1.15)	0.69 (0.51–0.94)	0.65 (0.46–0.91)	0.01
Model 2 HR (95% CI)[e]	1.0	0.76 (0.53–1.09)	0.69 (0.51–0.95)	0.68 (0.48–0.96)	0.04
	Canned Tuna Fish (3–4 oz.)				
	<1/month	**1–3/month**	**1/week**	**>1/week**	
Disease-free survival[b]					
Events	85	113	127	61	
Model 1 HR (95% CI)[d]	1.0	1.07 (0.81–1.42)	1.12 (0.85–1.48)	1.34 (0.96–1.88)	0.08
Model 2 HR (95% CI)[e]	1.0	1.06 (0.80–1.41)	1.12 (0.85–1.48)	1.33 (0.95–1.86)	0.09
Overall survival					
Events	71	86	101	47	
Model 1 HR (95% CI)[d]	1.0	0.91 (0.65–1.27)	0.99 (0.71–1.36)	1.17 (0.78–1.75)	0.37
Model 2 HR (95% CI)[e]	1.0	0.90 (0.64–1.26)	1.02 (0.73–1.41)	1.23 (0.82–1.84)	0.23
	Other fish (3–5 oz.)				
	Never	**<1/month**	**1–3/month**	**≥1/week**	
Disease-free survival [b]					
Events	72	52	134	128	
Model 1 HR (95% CI)[d]	1.0	0.80 (0.56–1.14)	0.86 (0.64–1.14)	0.92 (0.69–1.23)	0.83

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	Never	<1/month	1–3/month	≥1/week	P_{trend} [b]
		Dark meat fish (3–5 oz.)[a]			
Model 2 HR (95% CI)[e]	1.0	0.78 (0.55–1.12)	0.83 (0.62–1.12)	0.86 (0.64–1.16)	0.54
Overall survival					
Events	58	40	103	104	
Model 1 HR (95% CI)[d]	1.0	0.98 (0.64–1.50)	0.91 (0.64–1.31)	1.03 (0.72–1.48)	0.87
Model 2 HR (95% CI)[e]	1.0	0.95 (0.62–1.46)	0.86 (0.60–1.24)	1.03 (0.71–1.48)	0.86
		Breaded fish cakes, pieces, or sticks (1 serving, store bought)			
	Never	**<1/month**	**1–3/month**	**≥1/week**	
Disease-free survival[b]					
Events	168	58	110	50	
Model 1 HR (95% CI)[d]	1.0	0.81 (0.60–1.10)	1.10 (0.87–1.40)	1.16 (0.84–1.60)	0.18
Model 2 HR (95% CI)[e]	1.0	0.77 (0.57–1.04)	1.05 (0.82–1.35)	1.10 (0.79–1.53)	0.35
Overall survival					
Events	136	43	82	44	
Model 1 HR (95% CI)[d]	1.0	0.88 (0.62–1.25)	1.09 (0.81–1.45)	1.10 (0.74–1.62)	0.45
Model 2 HR (95% CI)[e]	1.0	0.81 (0.57–1.16)	1.05 (0.78–1.41)	1.10 (0.74–1.64)	0.47
		Shrimp, lobster, scallops, clams as a main dish (1 serving)			
	Never	**<1/month**	**1–3/month**	**≥1/week**	
Disease-free survival[b]					
Events	95	55	147	89	
Model 1 HR (95% CI)[d]	1.0	0.84 (0.60–1.17)	1.10 (0.85–1.43)	1.08 (0.81–1.45)	0.38
Model 2 HR (95% CI)[e]	1.0	0.80 (0.57–1.12)	1.05 (0.81–1.37)	1.02 (0.76–1.38)	0.63
Overall survival					
Events	79	44	112	70	
Model 1 HR (95% CI)[d]	1.0	0.95 (0.65–1.39)	1.03 (0.75–1.41)	1.05 (0.74–1.50)	0.75
Model 2 HR (95% CI)[e]	1.0	0.92 (0.62–1.35)	0.97 (0.70–1.33)	1.04 (0.73–1.50)	0.87

[a] Examples of dark meat fish included on the food frequency questionnaire included mackerel, salmon, sardines, bluefish, swordfish.

[b] P_{trend} calculated by modeling the median of each category as a continuous term.

[c] Disease-free survival = time from the baseline food frequency questionnaire to tumor recurrence, occurrence of a new primary colon tumor, or death from any cause, whichever came first.

[d] Cox proportional hazards regression model adjusted for age, sex, and energy intake.

[e] Cox proportional hazards regression model adjusted for variables in Model 1 plus T-stage, number of positive lymph nodes, baseline performance status, treatment arm, body mass index, physical activity, smoking, and aspirin use.