Marine omega-3 fatty acid intake and survival of stage III colon cancer according to tumor molecular markers in NCCTG Phase III trial N0147 (Alliance)

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Abstract

Marine omega-3 polyunsaturated fatty acids (MO3PUFAs) have anticancer properties and may improve colon cancer survival. However, it remains unknown whether the benefit differs by tumor molecular subtype. We examined data from a phase III randomized trial of FOLFOX or FOLFOX + cetuximab among 1,735 stage III colon cancer patients who completed a dietary questionnaire at enrollment. Multivariable hazard ratios and 95% confidence intervals (CIs) were calculated for the association between MO3PUFA and disease-free survival (DFS) and overall survival according to *KRAS* and *BRAF*^{V600E} mutations and DNA mismatch repair (MMR) status. Higher MO3PUFA intake was associated with improved 3-year DFS for *KRAS*-wildtype tumors (77% vs. 73%; HR, 0.84, 95% CI, 0.67-1.05), but not *KRAS*-mutant tumors (64% vs. 70%; HR, 1.30, 95% CI, 0.97-1.73; $P_{interaction}$=0.02). Similar heterogeneity was found by MMR ($P_{interaction}$=0.14): higher MO3PUFA was associated with better 3-year DFS for tumors with deficient MMR (72% vs. 67%), but not proficient MMR (72% vs. 72%). No heterogeneity was found by *BRAF*^{V600E} mutation. Similar findings were obtained for overall survival. In conclusion, we found a suggestive beneficial association between higher MO3PUFA intake and improved survival among stage III colon cancer patients with wildtype *KRAS* and deficient MMR. Given the relatively small number of cases with tumor molecular assessments, further studies, preferably through pooled analyses of multiples cohorts, are needed to validate our findings.

Keywords

colorectal cancer; survivorship care; nutrition; inflammation; tumor microenvironment

Introduction

Colorectal cancer (CRC) is the third most common cancer and third leading cause of cancer death in the United States.[1] Currently, there are more than 1.4 million Americans living with colorectal cancer.[2] Substantial data support that marine ω-3 polyunsaturated fatty acids (MO3PUFAs) (i.e., eicosapentaenoic acid [EPA], docosahexaenoic acid [DHA] and docosapentaenoic acid [DPA]) have potential anti-cancer effects and may improve CRC survival through modulation of local and systemic immune response.[3] Consistent with the mechanistic data, we recently reported that higher intake of MO3PUFA after diagnosis was associated with better survival among patients with established CRC in two independent cohort studies.[4, 5] Furthermore, a phase II randomized placebo-controlled trial showed that

preoperative treatment with MO3PUFA conferred survival benefits among patients with CRC liver metastases.[6] These data indicate the potential of MO3PUFA as an adjuvant treatment agent for improving CRC survival.

Although traditionally considered as a single disease, CRC is now known as a heterogeneous entity characterized by distinct molecular markers.[7] Congruent with this notion, we found that the beneficial association between higher intake of MO3PUFA and lower incidence of CRC was restricted to a subset of tumors with microsatellite instability (MSI).[8] MSI is present in about 15% of CRC cases and caused by the loss of DNA mismatch repair (MMR) activity.[9] In line with our observation and the anti-inflammatory properties of MO3PUFA, growing data support the critical role of inflammation and dysregulated antitumor immune response in the development of MSI tumors.[10] Immune checkpoint inhibitor therapy has been shown to be more effective for treating cancers with MSI.[11, 12] These data suggest that high intake of MO3PUFA after diagnosis may primarily benefit patients with MSI tumors.

Besides MSI, other molecular markers have also been characterized in CRC. *KRAS*, a member of the rat sarcoma virus (*ras*) gene family of oncogenes, is a central signaling node that controls transcription of genes important for cell metabolism, growth and proliferation. [13] Somatic mutations in *KRAS* codons 12 and 13 are found in approximately 40% colon cancer cases and predict the resistance to epidermal growth factor receptor (EGFR) antibody therapy.[14] *BRAF* is another important oncogene in colon cancer that acts downstream from *KRAS*. A point mutation in *BRAF* (V600E), which is detected in approximately 8% of colon cancers, results in epigenetic silencing of the MMR pathway, thereby leading to the development of MSI and activation of the oncogenic pathway to promote cell proliferation and survival.[15, 16]

Given our prior data about MO3PUFA and MSI, as well as the role of *KRAS* and *BRAF* in predicting CRC treatment and survival, we examined the prognostic influence of MO3PUFA intake according to these markers in a phase III randomized adjuvant trial of stage III colon cancer patients (the Alliance N0147 trial). Our primary hypothesis was that MO3PUFA intake is associated with better survival and this beneficial association may be more evident for tumors with MSI.

Materials and Methods

Study population

The N0147 is a multicenter phase III trial led by the North Central Cancer Treatment Group (NCCTG) in which patients with resected stage III colon cancer were randomly assigned to treatment with infusional fluorouracil, leucovorin, and oxaliplatin (FOLFOX) with or without adjuvant cetuximab.[17] NCCTG is now part of the Alliance for Clinical Trials in Oncology. Eligible patients had histologically confirmed adenocarcinoma of the colon, at least one pathologically confirmed positive lymph node, complete surgical resection performed   56 days before random assignment, and Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2. Major exclusion criteria include evidence of metastatic disease, prior or concurrent malignancies, previous EGFR therapy, age younger than 18 years, and one or more of several exclusionary comorbid conditions at the time of

random assignment. A total of 2,686 patients were recruited and randomly assigned between February 10, 2004 and November 25, 2009. Given the emerging data indicating that patients with *KRAS* mutated tumors gained limited or no benefit from cetuximab treatment,[17] the study enrollment criteria was modified in early 2008 such that all participants were subsequently tested for somatic *KRAS* mutations before random assignment, and only those with *KRAS* wildtype tumors were randomly assigned to treatment arms per the original protocol. The current analysis is limited to participants enrolled onto the primary treatment comparison arms (FOLFOX vs. FOLFOX plus cetuximab) who had completed a dietary questionnaire at the baseline visit (n = 1,735; Figure 1). Each participant signed an institutional review board-approved, protocol-specific informed consent in accordance with federal and institutional guidelines.

Exposure assessment

Until a change in protocol in 2008, participants in N0147 were asked at study enrollment to complete a 68-item modified Block Brief food frequency questionnaire (FFQ) that inquired about their usual eating habits of each listed food and beverage during the past year, including average frequency (nine options ranging from never to every day) and amount (four options) or serving size (mostly four options based on the portion size pictures provided for each food). Daily intake for each nutrient was calculated by multiplying the reported frequency and amount of consumption of each item by its nutrient content and then summing across all foods. The nutrient content of the diet was calculated by NutritionQuest using the US Department of Agriculture Food and Nutrient Database for Dietary Studies, which provides a population-weighted nutrient composition for each food item based on national dietary intake data.[18]

For assessment of MO3PUFA intake, two fish items were asked on the FFQ, including *fried fish or fish sandwich and any other fish or shellfish not fried (including tuna)*. MO3PUFA included EPA, DHA, and DPA; with the mean intake of 0.020, 0.038, and 0.008 g/day, respectively. We adjusted MO3PUFA intake for total caloric intake using the nutrient residual method.[19] Fish consumption in grams per week was assessed by taking the product of average frequency of each item per week, number of grams in a medium serving, and serving size (0.5 for small, 1.0 for medium, and 1.5 for large). Total fish consumption was calculated by summing across the two fish items and examined in relation to survival outcomes in the secondary analysis. The FFQ has been validated using multiple dietary records as the reference, shown reasonable validity for macronutrients,[20] and used previously for fish and MO3PUFA assessment.[21, 22]

Covariate assessment

To control for potential confounding by other dietary factors, we also calculated total intake (including supplements) of vitamin D, calcium, fiber and processed red meat based on the FFQ. In addition, a lifestyle questionnaire was administered at the baseline study visit to collect detailed information on various potential predictors of cancer survival, including body weight, height, frequency of physical activity (at any, moderate, and vigorous level), smoking history, and use of aspirin and multivitamin. We calculated body mass index as weight in kilograms divided by height in meters squared.

Assessment of tumor molecular markers

Macrodissected formalin-fixed, paraffin-embedded tumor tissue from the original surgical resection were requested for all study participants and sent to the Mayo Clinic for centralized testing of DNA MMR status, and *KRAS* and *BRAF* mutations. All assays were performed in a Clinical Laboratory Improvement Amendments-compliant laboratory, using appropriate quality control procedures, and interpreted without knowledge of treatment, patient, and outcome information. DNA MMR status was determined by immunohistochemical assessment of three proteins: MLH-1, MSH-2, and MSH-6, as previously described.[23] Patients with tumors exhibiting a loss of protein expression for any of these markers were classified as having defective MMR (dMMR); patients with no loss of expression were classified as having proficient MMR (pMMR). DNA extracted from tumor specimens was used to mutation testing. Seven mutations in codons 12 and 13 of *KRAS* exon 2 (Gly12Ala, Gly12Asp, Gly12Arg, Gly12Cys, Gly12Ser, Gly12Val, and Gly13Asp) were tested using the DxS mutation test kit KR-03/04 (DxS, Manchester, UK). Tumors with any of the aforementioned *KRAS* mutations were classified as mutant *KRAS*, whereas the rest were classified as wildtype *KRAS*. Assessment for the *BRAF*[V600E] mutation was performed using a Mayo-developed multiplex allele-specific polymerase chain reaction–based assay.[24] After amplification, polymerase chain reaction products were analyzed on an ABI 3130xl instrument (Life Technologies, Applied Biosystems, Grand Island, NY) and scored for the presence or absence of the V600E variant only. Tumors with the *BRAF*[V600E] mutation were classified as mutant *BRAF* (vs wildtype).

Outcome ascertainment

The primary outcome of the study was disease-free survival (DFS), defined as the time from registration to the earliest occurrence of the first documented colon cancer recurrence or death as a result of any cause. The secondary endpoint was overall survival (OS), defined as the time from registration to death of any cause. Because the evaluation of recurrence and death became too sparse when the follow-up extended beyond what was specified in the protocol (5 years), we censored DFS and OS at 5 years and 8 years post-registration, respectively, on the basis of the consistency of available follow-up information.

Statistical analysis

Given the higher intake of MO3PUFA in men than women in this study (Table 1), we used sex-specific cutoffs to categorize MO3PUFA intake into quartiles. Basic characteristics of participants according to quartiles of MO3PUFA intake were compared using the Kruskal-Wallis[25] and chi-square tests[26] for continuous and categorical variables, respectively. We calculated the 3-year DFS and 5-year OS in each quartile of MO3PUFA intake using Kaplan-Meier method.[27] We used Cox proportional hazards regression models to calculate the hazard ratios (HRs) and 95% confidence intervals (CIs) of DFS and OS according to quartiles of MO3PUFA intake.[28] The linear trend of HRs across different quartiles was tested by including the median intake of each quartile in the model. Proportional hazards assumption was verified by testing for a nonzero slope of the scaled Schoenfeld residuals on ranked failure times.[29] To minimize the influence of potential confounding, we considered two models: model 1 was adjusted for age at diagnosis (continuous), sex (male, female), and

study arm (FOLFOX vs. FOLFOX plus cetuximab); model 2 was further adjusted for additional *a priori* selected predictors for CRC survival, including tumor location (distal and proximal colon, both), number of affected lymph nodes (1-3, >3), T stage (T1-T2, T3, T4), ECOG performance score (0, 1, 2), aspirin use (none, full-dose or extra-strength, low-dose or body-strength), MMR status (dMMR, pMMR), smoking status (never, past, current 20 cigarettes/day, current > 20 cigarettes/day), alcohol consumption (never, former, current), body mass index (< 18.5, 18.5 to 22.4, 22.5 to 24.9, 25 to 29.9, 30 kg/m^2), frequency of vigorous physical activity (never, monthly, weekly), multivitamin use (no, yes), and total intake of vitamin D, calcium, fiber, and processed red meat (continuous). For covariates with missing data, we created and included missing indicators in the multivariable model.

To examine whether the MO3PUFA-survival association differs by tumor molecular markers, we performed stratified analysis according to MMR status (dMMR, pMMR), and *KRAS* and *BRAF* mutations (wildtype, mutant). In light of the relation between *BRAF* mutation and MSI, we also stratified by the combined status of MMR and *BRAF*. Given the limited sample size in each stratum, we used the binary MO3PUFA intake (low and high intake groups) dichotomized at the sex-specific median levels. Multiplicative interaction was assessed using the Wald test for the product term between the stratified variable and MO3PUFA intake. To facilitate clinical interpretation, we also calculated the 3-year DFS and 5-year OS in each of the patient strata characterized jointly by MO3PUFA intake and the status of molecular markers.

As a secondary analysis, we examined the association of fish intake with DFS and OS using similar analytic approaches.

Analyses were based on follow-up time through August 5, 2015, and were performed by using SAS version 9.4 (SAS Institute, Cary, NC). Two-sided *P* values less than 0.05 were considered statistically significant. All data collection and statistical analyses were performed by the Alliance Statistics and Data Center.

Results

Median follow-up time was 6.9 years among the 1,735 patients included in this analysis. The median consumption of MO3PUFAs was 0.05 g/day in women [interquartile range (IQR): 0.03-0.08] and 0.06 g/day in men (IQR: 0.04-0.09). Table 1 presents the basic characteristics of the 1,735 patients according to quartiles of MO3PUFA intake. Compared to patients with lower MO3PUFA intake, those with higher intake were less likely to have proximal colon cancer, dMMR and *BRAF* mutation, and were more likely to have *KRAS* mutation. They also tended to use low-dose aspirin and multivitamins, to exercise, to consume more fiber and less processed red meat, and to refrain from smoking.

Table 2 shows the association of MO3PUFA intake with DFS and OS. No statistically significant association was found between MO3PUFA intake and overall DFS or OS (*P* for trend=0.84 and 0.73 for the multivariable analysis, respectively).

We then examined the stratified association of dichotomized MO3PUFA intake with survival according to tumor molecular markers (Table 3). We observed a statistically significant

interaction with *KRAS* status (*P* for interaction=0.02 for DFS); MO3PUFA intake above the median was associated with better 3-year DFS among patients with *KRAS*-wildtype tumors (77% vs. 73%; multivariable HR=0.84, 95% CI, 0.67-1.05), whereas no beneficial association was found for those with *KRAS*-mutant tumors (64% vs. 70%; HR=1.30, 95% CI, 0.97-1.73). Similar heterogeneity was found by MMR status: higher MO3PUFA intake was associated with better DFS for tumors with dMMR (72% vs. 67%; HR=0.69, 95% CI, 0.41-1.16), but not for those with proficient MMR (72% vs. 72%; HR=1.08, 95% CI, 0.89-1.30), although the formal test for interaction was not statistically significant (*P* for interaction=0.14). No difference in the MO3PUFA-survival association was observed by *BRAF* mutation. When tumors were classified jointly according to MMR and *BRAF* mutation status, higher MO3PUFA intake was suggestively associated with improved DFS for tumors with dMMR, regardless of *BRAF* mutation status (HR=0.47 [95% CI, 0.20-1.12] for dMMR/*BRAF* mutant tumors; 0.59 [95% CI, 0.25-1.38] for dMMR/*BRAF* wildtype tumors), although the associations did not achieve statistical significance due to the sparse data. Similar findings were obtained for OS.

In a sensitivity analysis, we examined the interactions between MO3PUFA intake and molecular markers with MO3PUFA intake categorized in quartiles instead of binary classification. Although the number of events was limited in some strata, the interaction pattern remained essentially unchanged. For example, the multivariable HRs of DFS comparing extreme quartiles of MO3PUFA intake were 0.83 (95% CI, 0.61-1.14) for *KRAS*-wildtype tumors and 1.43 (95% CI, 0.95-2.16) for *KRAS*-mutant tumors (data only shown in the text).

For the secondary analysis on fish intake, we observed similar patterns of interaction for DFS (*P* for interaction=0.01 for *KRAS* mutation, 0.40 for MMR). Higher fish intake was associated with better DFS in patients with *KRAS*-wildtype (HR=0.80, 95% CI, 0.64-1.00) and dMMR tumors (HR=0.85, 95% CI, 0.50-1.43), whereas no beneficial association was found for *KRAS*-mutant (HR=1.34, 95% CI, 1.00-1.80) and pMMR tumors (HR=1.02, 95% CI, 0.85-1.23) (Supplementary Table 1).

Discussion

In this prospective study of stage III colon cancer patients, we noted a suggestive beneficial association between higher MO3PUFA intake and improved DFS and OS among patients with wildtype *KRAS* and dMMR tumors. These data appear to be consistent with our previous findings that MO3PUFA was preferably associated with lower risk of MSI tumors, and together suggest that MO3PUFA may improve colon cancer patients' survival by modulation of the unique microenvironment in tumors that lack *KRAS* mutation and arise from the MSI pathway.

Increasing data support that there are at least two pathways for colorectal tumorigenesis[16]: the conventional pathway that originates from loss of adenomatous polyposis coli function followed by mutations in the oncogene *KRAS*, activation of prostaglandin-endoperoxide synthase 2 and loss of heterozygosity of *P53*; and the serrated pathway that is characterized by methylation-induced transcriptional silencing of the DNA MMR gene *MLH1* and the

resultant development of MSI tumors. Therefore, *KRAS* mutation and MSI may represent two largely distinct etiological pathways underlying CRC development, although a small subset of tumors demonstrates both features.

Chronic inflammation has been proposed to constitute risk factors for the development of MSI cancers,[9] which demonstrate a vigorous immune microenvironment characterized by high infiltration of activated cytotoxic T lymphocytes and upregulation of counter-inhibitory checkpoint molecules.[10, 30] Therefore, MSI tumors are more responsive to immune checkpoint-targeted therapy.[31] Mechanistically, chronic inflammation induces oxidative stress, a state in which reactive oxygen species are produced in excess abundance and modify DNA structures.[32] Moreover, key mediators in the inflammatory process, such as prostaglandin E_2, may promote MSI development by inducing transcriptional silencing of DNA-repair genes through DNA methylation-mediated mechanisms.[33]

In line with these clinical and mechanistic data, our previous study indicated that higher intake of MO3PUFA, a potent anti-inflammatory agent, might preferably protect against the development of MSI tumors.[8] We further showed that this benefit may be related to the improvement in the regulatory T cell-mediated immunosuppression through the anti-inflammatory activity of MO3PUFA.[34] The current study extends these findings and suggests that, in patients with established MSI tumors, high intake of MO3PUFA after diagnosis may also be beneficial and contributes to improved survival. Several clinical trials have indicated that MO3PUFA supplementation reduces circulating and colonic mucosal levels of pro-inflammatory mediators, including prostaglandin E_2 and chemokine C-C motif ligand 2,[35–38] improves immune response,[39] and delay tumor progression.[40] These findings support our results and may have important clinical implications. While newly approved immunotherapeutic agents for treating MSI tumors has shown great promise, the clinical efficacy remains suboptimal, with a response rate of 30-50% in the recent phase II trials. [11, 12] Therefore, given the well-known immunomodulatory activity and the observed benefit of MO3PUFA for MSI tumors in our studies, it is possible that integrating MO3PUFA into the current immunotherapeutic regimen may help improve treatment outcome.[41]

For *KRAS* mutation, we found that the protective association of MO3PUFA intake with colon cancer survival was restricted to *KRAS*-wildtype tumors. This is not surprising, since increasing data suggest that *KRAS*-mutant tumors are largely driven by metabolic dysregulation rather than inflammation.[42] Activation of oncogenic KRAS plays an important role in metabolic reprogramming of tumor cells and drives the shift toward an anabolic metabolism necessary to produce biomass to support unconstrained proliferation.[43–45] Consistent with the crucial role of KRAS in metabolic regulation,[42, 46] colorectal tumor subtype with overrepresentation of *KRAS* mutation (the so-called consensus molecular subtype 3) is found to be enriched with multiple metabolism signatures in an international consortium for gene expression profiling of colon cancer.[7] Therefore, it is possible that the predominance of the immunomodulatory activities of MO3PUFA may make it more effective for improving the tumor microenvironment and clinical outcomes of *KRAS*-wildtype tumors that have fewer metabolic dependencies.

In contrast with the positive findings in the tumor marker-stratified analysis, we did not find any association between higher MO3PUFA intake and survival in the overall cohort. This observation contrasts with our previous reports that higher intake of MO3PUFA after diagnosis was associated with better survival in two independent cohorts that include stage I-III CRC[4] and stage III colon cancer only,[5] respectively. One of the reasons may be related to the brief FFQ used in the current study, which included only two questions about fish intake and did not specifically inquire about dark-meat fish (e.g., mackerel, salmon, and sardines) that contains much higher MO3PUFA and is the major source of MO3PUFA intake in the US.[47, 48] As a consequence, MO3PUFA intake assessed in the current study was much lower than in our prior study that is also based on a chemotherapy trial of stage III colon cancer patients[5] (median intake in the highest quartile: 0.13 vs. 0.40 g/day). Also, as diet was assessed at baseline only, we were unable to capture any change in patients' diet during or after treatment. The resultant measurement error from these sources may have attenuated any association between MO3PUFA intake and patients' survival in the overall cohort, highlighting the need for better assessment of MO3PUFA status in future studies (e.g., using the plasma samples).

Another limitation of the current study is the increased likelihood for chance findings due to multiple hypothesis testing and limited sample size in some of the strata. However, all the tested hypotheses were developed *a priori* based on the previous evidence. Furthermore, consistent findings obtained in the current and prior studies and the supporting mechanistic data indicate the plausibility of our observations. In addition, given that this analysis is a secondary analysis of a clinical trial, residual confounding cannot be excluded. However, the standardized treatment in the context of a chemotherapy trial minimized any confounding by treatment, especially considering that the parent study reported a null effect. Moreover, we adjusted for a variety of covariates that may influence cancer survivorship and the results appeared to be robust. Nonetheless, further larger studies are needed to confirm the findings.

Our study has several strengths. It represents one of the first efforts to prospectively characterize the prognostic influence of a dietary factor according to tumor molecular profiles. The findings not only provide insight into the biological mechanisms underlying the anti-tumor effect of MO3PUFA, but also have clinical implications for development of precision therapy.[49] Moreover, systematic collection of tumor tissues and centralized assessment of tumor markers using standard protocols ensure the data quality and minimize any outcome misclassification. Finally, the availability of detailed information on potential confounders with detailed outcome follow-up allows for robust confounding control and minimizes selection bias.

In conclusion, higher intake of MO3PUFA may be associated with better survival in stage III colon cancer patients with wildtype *KRAS*. A suggestive beneficial association is also found for tumors with deficient MMR. The collective findings of the current and prior studies suggest a potential benefit of MO3PUFA in modulation of the inflammatory microenvironment in tumors arising from the MSI pathway. However, given that individual studies like ours are limited by the relatively small number of cases with tumor molecular assessments, further studies, preferably through pooled analyses of multiples cohorts, are needed to further validate our findings. Also, future studies using circulating MO3PUFA

levels would provide important data to help elucidate the role of MO3PUFA in colon cancer survival.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements:

Ethics approval and consent to participate

The study was approved by Mayo Clinic Institutional Review Board and NCCTG (now part of Alliance for Clinical Trials in Oncology). Each participant of the study signed an institutional review board-approved, protocol-specific informed consent in accordance with federal and institutional guidelines. The study was performed in accordance with the Declaration of Helsinki.

Abbreviations:

CI	confidence interval
CRC	colorectal cancer
DFS	disease-free survival
DHA	docosahexaenoic acid
dMMR	defective mismatch repair
DPA	docosapentaenoic acid
ECOG	Eastern Cooperative Oncology Group
EGFR	epidermal growth factor receptor
EPA	eicosapentaenoic acid
FFQ	food frequency questionnaire
HR	hazard ratio
IQR	interquartile range
MMR	mismatch repair
MO3PUFA	marine omega-3 polyunsaturated fatty acid

MSI	microsatellite instability
NCCTG	North Central Cancer Treatment Group
OS	overall survival
pMMR	proficient mismatch repair.

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Novelty & Impact Statements

In by far the largest study, we found a suggestive association between higher consumption of marine omega-3 polyunsaturated fatty acid (MO3PUFA) and better colon cancer survival among patients with wild-type *KRAS* and deficient DNA mismatch repair. Taken together with our previous findings that higher MO3PUFA might lower risk of microsatellite instable colon cancer, these results suggest a benefit of MO3PUFAs for tumors arising from the microsatellite instability pathway, and have implications for improving patient care.

Total patients randomly assigned in N0147
(N=2686)

Excluded: n=951
- Patients enrolled following implementation of
  Addendum 9 (August 18, 2008): n=563
- Patients did not complete the Block Brief food
  frequency questionnaire (FFQ): n=144
- Patients did not complete patient questionnaire: n=76
- Patients with missing data on MO3PUFA intake: n=2
- Patients with implausible energy intake: n=109
- Patients with missing sex: n=4
- FFQ completed after first-cycle of treatment: n=53

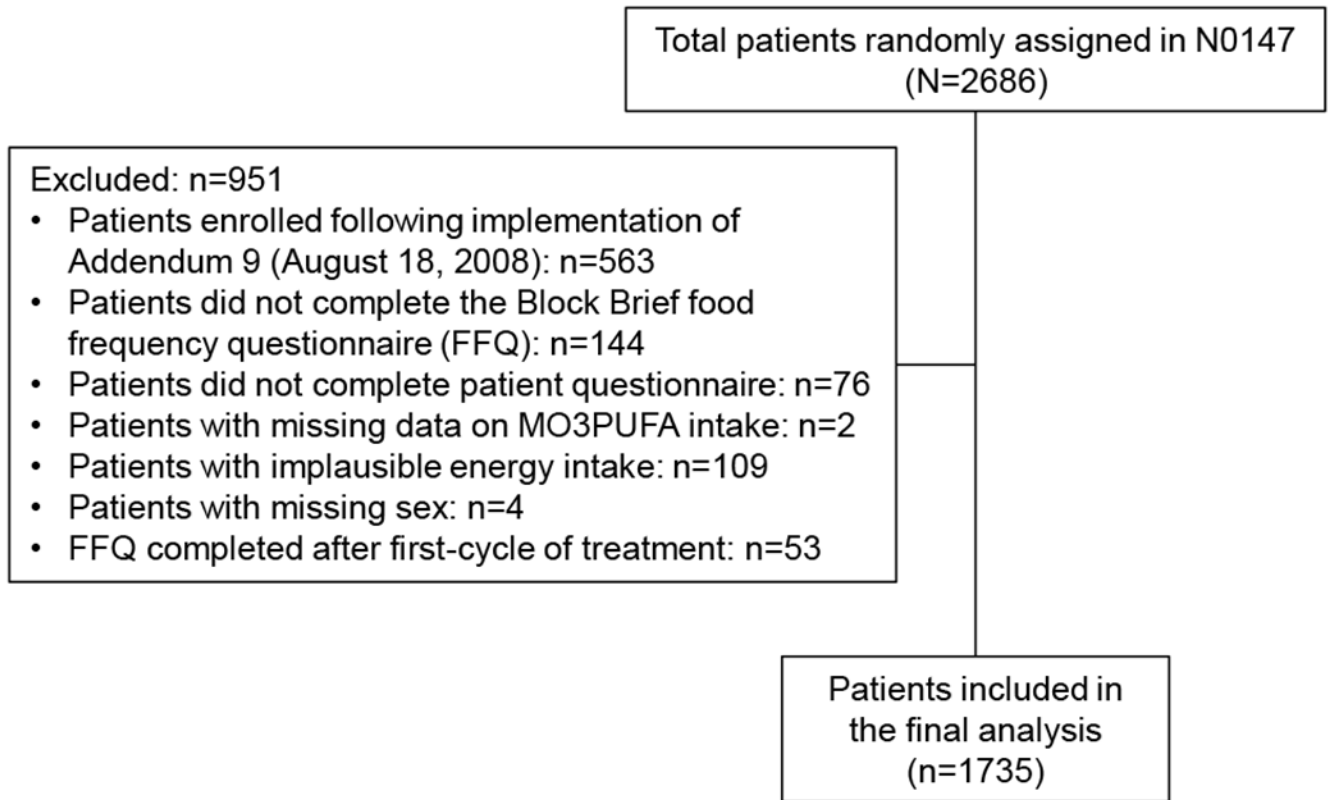Patients included in
the final analysis
(n=1735)

Figure 1.
Flowchart of the analytic cohort in North Central Cancer Treatment Group Phase III Trial
N0147

Table 1.

Basic characteristics of study participants according to post-diagnostic marine ω-3 polyunsaturated fatty acid (MO3PUFA) intake [*]

Variable	Quartile 1	Quartile 2	Quartile 3	Quartile 4
No. of participants	445	425	437	428
Age, year	58.2±12.0	57.2±11.9	58.5±10.6	58.1±10.6
Sex				
Women	216 (49)	205 (48)	212 (49)	205 (48)
Men	229 (51)	220 (52)	225 (51)	223 (52)
Study arm				
FOLFOX	219 (49)	205 (48)	212 (49)	214 (50)
FOLFOX plus cetuximab	226 (51)	220 (52)	225 (51)	214 (50)
Tumor subsite				
Proximal colon	236 (53)	238 (56)	220 (50)	207 (48)
Distal colon	206 (46)	179 (42)	205 (47)	219 (51)
Both	3 (1)	7 (2)	12 (3)	2 (1)
No. of affected nodes				
1-3	261 (59)	249 (59)	253 (58)	246 (57)
4	184 (41)	176 (41)	184 (42)	182 (43)
Histology				
High	117 (26)	100 (24)	118 (27)	105 (25)
Low	328 (74)	325 (76)	319 (73)	323 (75)
MMR Status				
pMMR	375 (88)	351 (86)	358 (86)	369 (89)
dMMR	49 (12)	56 (14)	58 (14)	48 (11)
BRAF Mutation				
Mutant	66 (16)	51 (13)	58 (14)	54 (13)
Wildtype	348 (84)	342 (87)	344 (86)	349 (87)
KRAS Mutation				
Mutant	143 (33)	139 (34)	155 (37)	146 (36)
Wildtype	288 (67)	265 (66)	259 (63)	263 (64)
ECOG Performance Score				
0	340 (76)	316 (74)	327 (75)	340 (79)
1	103 (23)	103 (24)	106 (24)	85 (20)
2	2 (1)	6 (2)	4 (1)	3 (1)
Aspirin Use				
None	295 (73)	259 (68)	256 (65)	254 (68)
Low-dose	67 (17)	77 (20)	88 (22)	81 (22)
Full dose	40 (10)	47 (12)	49 (13)	39 (10)
Regular Multivitamin Use				
0=NO	231 (55)	223 (57)	201 (50)	209 (52)
1=YES	187 (45)	168 (43)	204 (50)	190 (48)

Variable	Quartile 1	Quartile 2	Quartile 3	Quartile 4
Smoking Frequency				
Never	198 (45)	213 (50)	215 (49)	208 (49)
Past	200 (45)	185 (44)	193 (44)	195 (46)
Current, 20 cigs/day	29 (7)	18 (4)	24 (6)	19 (4)
Current, >20 cigs/day	15 (3)	9 (2)	3 (1)	5 (1)
Alcohol Consumption				
Never	132 (30)	120 (28)	134 (31)	128 (30)
Past	144 (33)	120 (28)	126 (29)	123 (29)
Current	167 (37)	185 (44)	176 (40)	176 (41)
Vigorous physical activity				
Never	327 (74)	278 (66)	296 (68)	254 (60)
Monthly	91 (21)	115 (27)	100 (23)	127 (30)
Weekly	23 (5)	29 (7)	37 (9)	46 (10)
BMI, kg/m^2				
<18.5	6 (1)	3 (1)	9 (2)	3 (1)
18.5-22.4	55 (12)	49 (12)	59 (14)	47 (11)
22.5-24.9	72 (16)	68 (16)	63 (14)	59 (14)
25.0-29.9	166 (37)	160 (38)	152 (35)	165 (39)
30	145 (33)	142 (33)	154 (35)	152 (35)
Total MO3PUFA, g/day	0.025±0.008	0.045±0.007	0.067±0.01	0.13±0.006
Women	0.021±0.007	0.039±0.005	0.063±0.009	0.12±0.005
Men	0.028±0.009	0.049±0.006	0.071±0.008	0.14±0.006
EPA, mg/day	6±3	12±4	20±5	44±22
DHA, mg/day	15±5	27±5	39±7	72±29
DPA, mg/day	3±2	6±2	9±3	16±7
Total omega-3 PUFA, g/day	1.1±0.6	1.1±0.4	1.2±0.4	1.3±0.4
Total omega-6 PUFA, g/day	10.3±3.1	10.3±2.7	10.8±2.6	10.9±2.9
Vitamin D, IU/day	334±251	301±233	326±229	334±226
Calcium, mg/day	870±483	871±467	850±450	863±470
Total Fiber, g/day	23.4±9.9	25.1±8.7	25.7±8.6	28.0±10.0
Fish, g/day	3.5±2.4	8.1±4.1	14.4±5.6	35.7±22.0
Processed red meat, g/day	31.7±33.1	29.6±28.1	29.7±26.2	26.3±29.3

Abbreviations: EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid.

*
 Mean±SD is shown for continuous variables. N (%) is shown for categorical variables. For some variables, the number of participants across all categories does not sum to the total due to missing data.

Table 2.

Post-diagnostic marine ω-3 polyunsaturated fatty acid (MO3PUFA) intake and disease-free survival and overall survival among patients with stage III colon cancer

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*P* for trend[*]
Median intake (interquartile range), g/day	0.03 (0.02-0.03)	0.04 (0.04-0.05)	0.07 (0.06-0.08)	0.12 (0.10-0.14)	
Disease-free survival (DFS)					
No. of events/patients	121/363	113/344	116/353	114/351	
3-year survival (%)	70.7	72.8	71.8	73.3	
Model 1, HR (95% CI) [†]	1 (reference)	0.97 (0.75-1.25)	0.96 (0.74-1.24)	0.93 (0.72-1.20)	0.60
Model 2, HR (95% CI) [‡]	1 (reference)	0.99 (0.76-1.29)	0.99 (0.76-1.29)	0.97 (0.75-1.27)	0.84
Overall survival (OS)					
No. of events/patients	109/363	95/344	92/353	96/351	
5-year survival (%)	77.0	77.8	79.0	80.0	
Model 1, HR (95% CI) [†]	1 (reference)	0.92 (0.70-1.22)	0.83 (0.63-1.10)	0.89 (0.68-1.17)	0.43
Model 2, HR (95% CI) [‡]	1 (reference)	0.88 (0.67-1.17)	0.83 (0.62-1.10)	0.93 (0.70-1.23)	0.73

Abbreviation: CI, confidence interval; HR, hazard ratio.

[*] *P* for trend was calculated for overall colon cancer.

[†] Cox proportional hazards regression model adjusted for sex, age, and study arm (FOLFOX vs. FOLFOX plus cetuximab).

[‡] Further adjusted for tumor subsite (proximal colon, distal colon, both), No. of affected nodes (1-3, >3), T stage (T1-T2, T3, T4), ECOG performance score (0, 1, 2), aspirin use (none, full dose or extra-strength, low-dose or body-strength), MMR Status (pMMR, dMMR), smoking status (never, past, current (20 cigs/day), current (>20 cigs/day)), alcohol consumption (never, past, current), BMI (<18.5, 18.5-22.4, 22.5-24.9, 25.0-29.9, 30), frequency of vigorous physical activity (never, monthly, weekly), multivitamin use (no, yes), and total intake of vitamin D, calcium, fiber, and processed red meat (continuous).

Table 3.

Association between post-diagnostic marine ω-3 polyunsaturated fatty acid (MO3PUFA) intake and disease-free survival and overall survival according to tumor subsite and molecular markers among patients with stage III colon cancer*

	Disease-free survival (DFS)				Overall survival (OS)			
	No. of events/patients	3-year survival (%)	HR (95% CI)[†]	P for interaction[‡]	No. of events/patients	5-year survival (%)	HR (95% CI)[†]	P for interaction[‡]
KRAS mutation								
Mutant								
Low intake	91/282	70	1 (reference)		79/282	78	1 (reference)	
High intake	123/301	64	1.30 (0.97–1.73)	0.02	103/301	72	1.26 (0.92–1.71)	0.04
Wildtype								
Low intake	181/553	73	1 (reference)		154/553	77	1 (reference)	
High intake	146/522	77	0.84 (0.67–1.05)		122/522	84	0.85 (0.67–1.09)	
MMR status								
pMMR								
Low intake	231/726	72	1 (reference)		200/726	78	1 (reference)	
High intake	243/727	72	1.08 (0.89–1.30)	0.14	196/727	80	1.03 (0.84–1.27)	0.47
dMMR								
Low intake	40/105	67	1 (reference)		35/105	68	1 (reference)	
High intake	31/106	72	0.69 (0.41–1.16)		31/106	75	0.78 (0.46–1.34)	
BRAF mutation								
Mutant								
Low intake	44/117	68	1 (reference)		46/117	64	1 (reference)	
High intake	42/112	70	1.01 (0.64–1.58)	0.73	42/112	68	1.00 (0.64–1.57)	0.90
Wildtype								
Low intake	219/690	73	1 (reference)		179/690	80	1 (reference)	
High intake	222/693	73	1.02 (0.84–1.23)		179/693	81	1.04 (0.84–1.29)	
MMR/*BRAF* status								
pMMR/Mutant								
Low intake	21/64	70	1 (reference)		22/64	68	1 (reference)	
High intake	29/60	63	1.74 (0.90–3.35)	0.12	29/60	60	1.65 (0.87–3.16)	0.07
dMMR/Mutant								

	Disease-free survival (DFS)				Overall survival (OS)			
	No. of events/patients	3-year survival (%)	HR (95% CI) [†]	P for interaction [‡]	No. of events/patients	5-year survival (%)	HR (95% CI) [†]	P for interaction [‡]
Low intake	23/51	63	1 (reference)		24/51	55	1 (reference)	
High intake	13/50	76	0.47 (0.20–1.12)		13/50	75	0.42(0.17–1.02)	
pMMR/Wildtype								
Low intake	201/629	72	1 (reference)		168/629	79	1 (reference)	
High intake	204/633	73	1.02 (0.84–1.25)		161/633	81	1.01(0.81–1.27)	
dMMR/Wildtype								
Low intake	14/48	72	1 (reference)		9/48	80	1 (reference)	
High intake	17/54	68	0.59 (0.25–1.38)		17/54	74	0.93(0.34–2.58)	

Abbreviation: dMMR, deficient mismatch repair; CI, confidence interval; HR, hazard ratio; pMMR, proficient mismatch repair.

*
Only patients who had available data on stratified variables (i.e., tumor subsite and molecular markers) were included in the analysis. Low intake was defined as below the median in each sex, and high intake as equal to or above the median intake. The median intake of MO3PUFA was 0.05 g/day in women and 0.06 g/day in men.

[†] Multivariable HRs (95% CIs) were derived from model 2 described in Table 2.

[‡] Wald test was used to calculate the p value for the product term between the stratified variable and MO3PUFA intake (both binary) except for the analysis by the joint MMR/BRAF status, for which likelihood ratio test with 3 degrees of freedom was used to compare the models with and without the product terms between MO3PUFA intake and MMR/BRAF status (4 categories).