Associations Between Cigarette Smoking Status and Colon Cancer Prognosis Among Participants in North Central Cancer Treatment Group Phase III Trial N0147

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ABSTRACT

Purpose

By using data from North Central Cancer Treatment Group Phase III Trial N0147, a randomized adjuvant trial of patients with stage III colon cancer, we assessed the relationship between smoking and cancer outcomes, disease-free survival (DFS), and time to recurrence (TTR), accounting for heterogeneity by patient and tumor characteristics.

Patients and Methods

Before random assignment to infusional fluorouracil, leucovorin, and oxaliplatin (FOLFOX) or FOLFOX plus cetuximab, 1,968 participants completed a questionnaire on smoking history and other risk factors. Cox models assessed the association between smoking history and the primary trial outcome of DFS (ie, time to recurrence or death), as well as TTR, adjusting for other clinical and patient factors. The median follow-up was 3.5 years among patients who did not experience events.

Results

Compared with never-smokers, ever smokers experienced significantly shorter DFS (3-year DFS proportion: 70% v 74%; hazard ratio [HR], 1.21; 95% CI, 1.02 to 1.42). This association persisted after multivariate adjustment (HR, 1.23; 95% CI, 1.02 to 1.49). There was significant interaction in this association by *BRAF* mutation status ($P = .03$): smoking was associated with shorter DFS in patients with *BRAF* wild-type (HR, 1.36; 95% CI, 1.11 to 1.66) but not *BRAF* mutated (HR, 0.80; 95% CI, 0.50 to 1.29) colon cancer. Smoking was more strongly associated with poorer DFS in those with *KRAS* mutated versus *KRAS* wild-type colon cancer (HR, 1.50 [95% CI, 1.12 to 2.00] v HR, 1.09 [95% CI, 0.85 to 1.39]), although interaction by *KRAS* mutation status was not statistically significant ($P = .07$). Associations were comparable in analyses of TTR.

Conclusion

Overall, smoking was significantly associated with shorter DFS and TTR in patients with colon cancer. These adverse relationships were most evident in patients with *BRAF* wild-type or *KRAS* mutated colon cancer.

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INTRODUCTION

Cigarette smoking is associated with a modest increased risk of colorectal cancer (CRC), particularly after long durations or high levels of exposure.¹⁻⁷ Increasing evidence, however, indicates that this association differs by certain tumor characteristics. In particular, smoking appears to be most strongly associated with risk of CRC exhibiting high microsatellite instability (MSI-H)^{3,4,6,7} or *BRAF* mutations.^{3,8} Differences in the association between smoking and CRC risk according to such molecular features may suggest a

differential impact of tobacco exposure on specific pathways of colorectal carcinogenesis.

The relationship between smoking history and tumor characteristics in CRC may also have prognostic implications to the extent that such tumor characteristics are associated with survival. To date, the relatively few studies that have assessed the relationship between smoking and survival after CRC diagnosis have yielded inconsistent results. In an analysis of patients with stage III colon cancer enrolled onto a phase III clinical trial, McCleary et al⁹ reported no significant association between current or former smoking and disease-free survival (DFS)

or overall survival. Smaller studies have reported an increased risk of 30-day mortality,¹⁰ postoperative complications,¹¹ and cause-specific mortality following surgical resection¹² for smokers with CRC. Another recent analysis from a population-based cohort of individuals with incident CRC found that those who reported being current smokers around the time of diagnosis were significantly more likely to die from their disease than never-smokers (hazard ratio [HR], 1.30; 95% CI, 1.09 to 1.74).¹³ This adverse association was restricted to patients with MSI-H disease,¹³ which could suggest that smoking has an adverse impact on treatment response and promotes tumor progression via pathways specific to MSI-H CRC; however, to the best of our knowledge, no other studies have assessed interaction between tumor characteristics and smoking in relation to CRC survival. We addressed this knowledge gap by using data from a large, multicenter clinical trial of adjuvant chemotherapy for stage III colon cancer.

PATIENTS AND METHODS

Study Population

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.¹⁴ 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. Patients with evidence of metastatic disease, prior or concurrent malignancies, previous EGFR therapy, age younger than 18 years, or one of several exclusionary comorbid conditions at the time of random assignment were excluded from participation. Between February 10, 2004, and November 25, 2009, a total of 2,686 patients were randomly assigned as part of the trial. On the basis of emerging data indicating limited or no benefit from cetuximab in patients with KRAS mutated tumors,¹⁵ changes were made to the study enrollment criteria in early 2008 such that all participants were subsequently tested for somatic KRAS mutations before random assignment, and only participants with KRAS wild-type tumors were randomly assigned to treatment arms per the original protocol. Details of this change to the protocol have been reported elsewhere.¹⁴ This analysis is limited to patients enrolled onto the primary treatment comparison arms (FOLFOX v FOLFOX plus cetuximab) who had completed a patient questionnaire at the baseline study visit ($n = 1,968$; Fig 1). Each participant signed an institutional review board-approved, protocol-specific informed consent in accordance with federal and institutional guidelines.

Exposure Ascertainment

Until a change in protocol in 2008, participants in N0147 were asked at study enrollment to complete a questionnaire eliciting information on family history of cancers, use of common medications, and risk behaviors, including smoking history. Specifically, participants were asked to report if they had ever been a regular cigarette smoker (defined as smoking ≥ 100 cigarettes in one's lifetime) and, if yes, whether they were currently (at enrollment) a smoker. Participants who reported having ever been a smoker were asked to report the age at which they began and, if applicable, quit regular smoking and the average number of cigarettes smoked per day.

Tumor Characterization

Colon tumor tissue blocks from the original surgical resection were obtained for all study participants and sent to the Mayo Clinic for centralized KRAS mutation testing and additional molecular analyses. DNA isolated from tumor specimens was used to test for seven mutations in codons 12 and 13 of KRAS exon 2 (Therascreen; DXS, Manchester, United Kingdom) and to test for the BRAF V600E mutation, as previously described.¹⁶ DNA mismatch repair (MMR) status was determined by immunohistochemical assessment of three proteins: hMLH-1, hMSH-2, and hMSH-6.¹⁷ Patients with tumors ex-

Fig 1. Derivation of translational analytic cohort in North Central Cancer Treatment Group Phase III Trial N0147.

hibiting 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). Assays for tumor characterization were interpreted without knowledge of treatment, patient, and outcome information.

Survival Outcomes

The primary clinical end point for this analysis was DFS. DFS was defined as the time from surgery to the earliest occurrence of the first documented colon cancer recurrence or death as a result of any cause. Associations were also assessed with respect to time to recurrence (TTR), in which TTR was defined as the time from surgery to first documented disease recurrence, and participants who died before any recurrence were censored at the time of their last study visit. On the basis of the consistency of available follow-up information, both outcomes were censored at 4 years postsurgery.

Statistical Analysis

Descriptive statistics were tabulated by smoking status and compared between groups by Kruskal-Wallis¹⁸ and χ^2 ¹⁹ tests for continuous and categorical factors, respectively. The distributions of survival times by smoking history were assessed by using Kaplan-Meier methods²⁰ and log-rank tests.²¹ Cox regression models²² were used to calculate HRs with associated 95% CIs for associations between time-to-event outcomes and multiple smoking parameters: history of ever smoking (ever smoker, never-smoker), current smoking status at the time of enrollment (never, former, current), age at smoking initiation (< 20 , ≥ 20 years), duration of smoking interval (ie, age at cessation [former smoker] or current age [current smoker] minus age at initiation; less than 10, 10 to 19, 20 to 29, 30 to 39, ≥ 40 years), packs smoked per day (never-smoker, ≤ 0.5 packs per day, > 0.5 to 1, > 1 to 1.5, > 1.5 to 2, > 2 packs per day), and time since smoking cessation (never-smoker, current smoker, < 10 years, ≥ 10 years). In multivariable Cox proportional hazards regression models, these associations were evaluated with adjustment for the following factors selected a priori: tumor subsite (distal, proximal), lymph node involvement (one to three, four or more nodes involved), T stage (T1 to T2, T3, T4), MMR status (pMMR, dMMR), BRAF mutation status (wild-type, mutated), ECOG performance score (0, 1, 2), age at diagnosis (continuous), sex (male, female), self-reported frequency of vigorous physical activity (never, monthly, weekly), body mass index (< 20 , 20 to 24.9, 25 to 29.9, ≥ 30 kg/m²), and alcohol consumption (never, former, current) at enrollment. Information on study arm was not included in the adjusted model, given previous reports of

no difference in DFS according to receipt of cetuximab in the clinical trial¹⁴ and no difference in the distribution of treatment according to smoking status. Tests for interaction with history of ever smoking were conducted with respect to sex, age, T stage, lymph node involvement, tumor subsite, *KRAS* mutation status, *BRAF* mutation status, and MMR status by using categorizations listed earlier, except for age (≤ 50 , > 50 years). Proportional hazards assumptions were verified by testing for a nonzero slope of the scaled Schoenfeld residuals on ranked failure times.²³

Analyses were based on follow-up time through September 7, 2011, and were performed by using SAS version 9.2 (SAS Institute, Cary, NC). Two-sided *P* values less than .05 were considered statistically significant. All data collection and statistical analyses were performed by the Alliance Statistics and Data Center.

RESULTS

Characteristics of the study population are presented in Table 1 according to smoking status. Compared with never-smokers, participants who were former or current smokers were older and were more likely to be male, to have colon tumors that were dMMR and/or *BRAF* mutated, to report no regular vigorous physical activity, and to be current alcohol consumers (all *P* < .01).

The median follow-up time was 3.5 years. Overall, DFS was significantly shorter in ever versus never-smokers (HR, 1.23; 95% CI, 1.02 to 1.49; *P* = .03; Table 2). This association with poorer DFS was somewhat stronger for current (HR, 1.47; 95% CI, 1.04 to 2.09) than for former smokers (HR, 1.20; 95% CI, 0.99 to 1.46). Associations with patterns of packs per day, duration of smoking interval, and time since smoking cessation were not statistically significant. However, results did suggest that DFS was poorest in individuals who smoked more than 30 cigarettes per day (ie, > 1.5 packs per day) and those who began smoking at age ≥ 20 years. Patterns of association were similar with respect to TTR (Table 2).

There was evidence of significant interaction between smoking and *BRAF* mutation status (interaction *P* = .03) in relation to DFS (Figs 2 and 3). Analyses stratified by *BRAF* mutation status indicated that the observed poorer DFS in ever versus never-smokers was limited to participants with *BRAF* wild-type tumors (HR, 1.36 [95% CI, 1.11 to 1.66] v HR, 0.80 [95% CI, 0.50 to 1.29] in those with *BRAF* mutated tumors). Although interaction was not statistically significant, there was also some suggestion of a difference in the association between smoking and DFS by *KRAS* mutation status: among participants with *KRAS* mutated disease, ever smokers experienced significantly poorer DFS than never-smokers (HR, 1.50; 95% CI, 1.12 to 2.00), whereas no association was evident in participants with *KRAS* wild-type disease (HR, 1.09; 95% CI, 0.85 to 1.39; interaction *P* = .07; Figs 2 and 3). In analyses stratified by *BRAF* or *KRAS*, differences in associations were most pronounced for comparisons of DFS in current versus never-smokers (HR_{*BRAF* wild-type}, 1.60 [95% CI, 1.10 to 2.32]; HR_{*BRAF* mutated}, 0.82 [95% CI, 0.29 to 2.35]; HR_{*KRAS* wild-type}, 1.08 [95% CI, 0.65 to 1.77]; HR_{*KRAS* mutated}, 2.30 [95% CI, 1.40 to 3.77]). Smoking was also associated with significantly shorter TTR in patients with *BRAF* wild-type (HR, 1.27; 95% CI, 1.11 to 1.70) or *KRAS* mutated disease (HR, 1.57; 95% CI, 1.17 to 2.12) but was not associated with TTR in patients with *BRAF* mutated (HR, 0.69; 95% CI, 0.42 to 1.15) or *KRAS* wild-type disease (HR, 1.03; 95% CI, 0.79 to 1.33; interaction *P* = .01 and *P* = .02 by *BRAF* and *KRAS* mutation status, respectively; data not shown).

Table 1. Characteristics of Study Participants From North Central Cancer Treatment Group Phase III Trial N01477 According to Self-Reported Cigarette Smoking History at the Time of Enrollment

Characteristic	Cigarette Smoking Status								P
	Never-Smokers (n = 931)				Ever Smokers (n = 1,028)				
	No.	%	Mean	SD	No.	%	Mean	SD	
Age, years			56.6	11.7			59.4	10.5	< .001
Sex									
Male	434	47			590	57			< .001
Female	497	53			438	43			
Study arm									
FOLFOX	463	50			513	50			.94
FOLFOX plus cetuximab	468	50			515	50			
Tumor subsite									
Distal colon	449	48			465	45			.21
Proximal colon	471	51			547	53			
Both	9	1			16	2			
Missing	2				0				
No. of affected nodes									
1-3	539	58			613	60			.44
> 3	392	42			415	40			
T stage									
T1-T2	137	15			145	14			.44
T3	695	75			763	74			
T4	98	11			120	12			
Missing	1				0				
MMR status									
pMMR	807	90			838	85			.001
dMMR	87	10			146	15			
Missing	37				44				
BRAF mutation status									
Wild type	755	89			811	84			.006
Mutated	98	11			154	16			
Missing	78				63				
KRAS mutation status									
Wild type	559	64			651	66			.20
Mutated	320	36			342	34			
Missing	52				35				
ECOG performance score									
0	737	79			760	74			.02
1	185	20			260	25			
2	9	1			8	1			
Frequency of vigorous physical activity									
Never	591	64			719	71			< .001
Monthly	253	27			231	23			
Weekly	84	9			67	7			
Missing	3				11				
Body mass index, kg/m ²									
< 20	30	3			46	4			.06
20-24.9	222	24			254	25			
25.0-25.9	327	35			392	38			
≥ 30.0	350	38			331	32			
Missing	2				5				
Alcohol consumption									
Never	413	45			184	18			< .001
Former	203	22			375	37			
Current	310	33			466	45			
Missing	5				3				

NOTE. Excludes nine participants with missing smoking history. Abbreviations: dMMR, defective mismatch repair; ECOG, Eastern Cooperative Oncology Group; FOLFOX, infusional fluorouracil, leucovorin, and oxaliplatin; MMR, mismatch repair; pMMR, proficient MMR; SD, standard deviation.

Table 2. Cigarette Smoking History, DFS, and TTR in Patients With Stage III Colon Cancer (North Central Cancer Treatment Group phase III trial N0147)

Table 2. Cigarette Smoking History, DFS, and TTR in Patients With Stage III Colon Cancer (North Central Cancer Treatment Group phase III trial N0147)														
Variable	No.	%	DFS						TTR					
			Unadjusted HR	95% CI	LR <i>P</i>	Multivariate-Adjusted HR	95% CI	LR <i>P</i>	Unadjusted HR	95% CI	LR <i>P</i>	Multivariate-Adjusted HR	95% CI	LR <i>P</i>
Smoking status														
Never-smoker	931	48	1.0 (ref)				1.0 (ref)				1.0 (ref)			
Ever smoker	1,028	53	1.21	1.02 to 1.42	.03	1.23	1.02 to 1.49	.03	1.17	0.98 to 1.39	.08	1.23	1.01 to 1.49	.04
Former smoker	888	45	1.19	1.00 to 1.41	.08	1.20	0.99 to 1.46	.04	1.15	0.95 to 1.38	.15	1.19	0.97 to 1.46	.07
Current smoker	140	7	1.31	0.95 to 1.82		1.47	1.04 to 2.09		1.33	0.95 to 1.86		1.47	1.03 to 2.11	
Missing	9													
No. of cigarettes per day														
Never-smoker	931	48	1.0 (ref)			.21	1.0 (ref)			.27	1.0 (ref)			.34
1-10	330	17	1.13	0.89 to 1.43		1.23	0.96 to 1.58		1.11	0.87 to 1.43		1.20	0.92 to 1.56	
11-20	387	20	1.21	0.97 to 1.51		1.29	1.02 to 1.65		1.18	0.93 to 1.48		1.28	1.00 to 1.66	
21-30	190	10	1.23	0.92 to 1.63		1.17	0.84 to 1.62		1.16	0.85 to 1.57		1.15	0.81 to 1.62	
31-40	70	4	1.47	0.97 to 2.21		1.35	0.86 to 2.12		1.53	1.00 to 2.33		1.53	0.97 to 2.40	
> 40	39	2	1.47	0.87 to 2.48		1.40	0.80 to 2.44		1.39	0.80 to 2.43		1.36	0.74 to 2.47	
Missing	21													
Age at smoking initiation (years)														
Never-smoker	931	48	1.0 (ref)			.12	1.0 (ref)			.04	1.0 (ref)			.26
≥ 20	279	15	1.26	0.99 to 1.60		1.39	1.08 to 1.80		1.17	0.90 to 1.52		1.32	1.01 to 1.75	
< 20	681	36	1.15	0.96 to 1.39		1.18	0.96 to 1.46		1.16	0.95 to 1.41		1.20	0.96 to 1.49	
Missing	77													
Time since smoking cessation (years)														
Never-smoker	931	49	1.0 (ref)			.17	1.0 (ref)			.07	1.0 (ref)			.28
≥ 10	585	31	1.20	0.99 to 1.46		1.24	1.00 to 1.55		1.16	0.95 to 1.48		1.22	0.97 to 1.54	
< 10	248	13	1.13	0.87 to 1.47		1.16	0.88 to 1.55		1.12	0.85 to 1.42		1.17	0.87 to 1.58	
Current smoker	140	7	1.31	0.95 to 1.81		1.51	1.07 to 2.14		1.33	0.95 to 1.86		1.50	1.04 to 2.15	
Missing	64													
Duration of smoking interval (years)*														
Never-smoker	931	50	1.0 (ref)			.56	1.0 (ref)			.26	1.0 (ref)			.69
< 10	146	8	1.15	0.83 to 1.59		1.36	0.96 to 1.19		1.15	0.82 to 1.62		0.35	0.94 to 1.94	
10-19	230	12	1.12	0.86 to 1.47		1.11	0.82 to 1.49		1.09	0.82 to 1.46		1.07	0.78 to 1.47	
20-29	199	11	1.19	0.90 to 1.58		1.33	0.99 to 1.80		1.22	0.91 to 1.63		1.36	0.99 to 1.86	
30-39	205	11	1.27	0.96 to 1.66		1.30	0.96 to 1.75		1.21	0.91 to 1.61		1.27	0.92 to 1.75	
≥ 40	149	8	1.12	0.81 to 1.55		1.15	0.81 to 1.63		1.10	0.79 to 1.55		1.17	0.80 to 1.69	
Missing	108													

NOTE. Adjusted for tumor site, number of involved lymph nodes, T stage, MMR status, performance score, physical activity, body mass index, alcohol consumption, age, and sex.

Abbreviations: DFS, disease-free survival; HR, hazard ratio; LR, log rank; ref, reference category; TTR, time to recurrence.

*Duration defined as the age at smoking cessation (former smoker) or age at reference (current smoker) minus the age at smoking initiation.

There was no evidence of interaction in associations between smoking history and DFS by sex, age, T-stage, number of affected nodes, tumor subsite, or MMR status, although numbers in some strata were limited (Fig 2). Patterns of association between smoking and TTR across these strata were similar to associations with DFS (not shown).

DISCUSSION

In this cohort of clinical trial participants with stage III colon cancer, we found that cigarette smokers experienced significantly shorter DFS and TTR than never-smokers. This adverse association with smoking differed according to tumor characteristics. In particular, there was evidence of significant interaction in the association between smoking

and DFS by *BRAF* mutation status, with observed associations limited to patients with *BRAF* wild-type disease. There was also suggestive evidence of interaction by *KRAS* mutation status, such that smoking was most strongly adversely associated with DFS in patients with *KRAS* mutated disease.

Overall, results presented here are consistent with findings from two recent studies assessing the relationship between prediagnostic smoking and prognosis after colon cancer diagnosis.^{9,13} As in this analysis, McCleary et al⁹ evaluated the relationship between smoking and DFS in a phase III clinical trial of stage III colon cancer (Cancer and Leukemia Group B 89803). Authors of that study found that current smokers (assessed midway through adjuvant therapy) had a nonsignificant 1.30-fold (95% CI, 0.93-fold to 1.84-fold) increased risk of disease recurrence or death relative to never-smokers; however,

Fig 2. Comparison of disease-free survival in ever versus never cigarette smokers according to patient and tumor characteristics. Adjusted for tumor site, nodal status, T stage, mismatch repair (MMR) status, performance score, physical activity, body mass index, alcohol consumption, age, and sex. Analyses among participants with T1-T2 stage disease not shown because of small numbers. dMMR, defective MMR; HR, hazard ratio; pMMR, proficient MMR.

this finding was attenuated in multivariable adjusted analyses (HR, 1.10; 95% CI, 0.73 to 1.64).⁹ Results from a population-based study of prediagnostic smoking and CRC survival indicated a somewhat stronger 1.51-fold (95% CI, 1.24-fold to 1.83-fold) increased risk of all-cause mortality and a 1.30-fold (95% CI, 1.09-fold to 1.74-fold) increased risk of cause-specific mortality in current versus never-smokers.¹³ Other studies, however, have suggested that prediagnostic smoking is not associated with colon cancer outcomes.^{24,25}

Observed inconsistencies in the literature regarding the association between smoking and colon cancer outcomes may be due, in part, to heterogeneity in this association by patient and tumor characteristics. For example, our finding that smoking was not associated with DFS or TTR among patients with *BRAF* mutated or *KRAS* wild-type colon cancer suggests that studies conducted in settings in which these tumor characteristics are more prevalent might yield more modest findings. Accordingly, we found no significant association between smoking and colon cancer DFS among women, patients diagnosed at age ≥ 50 years, and patients with dMMR tumors, all of whom are more likely to have *BRAF* mutated disease.²⁶⁻²⁹

Consistent with our general findings of heterogeneity in the association between smoking and colon cancer outcomes by tumor characteristics, one previous study reported that the adverse association between smoking and overall survival was significantly stronger in patients with MSI-H CRC (HR, 3.40; 95% CI, 1.59 to 7.29) than in those with microsatellite stable (MSS) disease (HR, 1.33; 95% CI, 1.03 to 1.70; $P_{\text{interaction}} = .02$).¹³ Although we did not evaluate associations with smoking by MSI status, we conversely found that the association between smoking and colon cancer DFS was null or more modest in

patient groups with characteristics associated with MSI-H disease (ie, older age,^{8,30} female sex,^{8,30,31} *BRAF* mutated disease^{28-30,32}). We analyzed associations on the basis of MMR protein expression patterns, which may be viewed as a proxy for MSI status, given that almost all MSI-high tumors are dMMR and almost all MSS tumors are pMMR¹⁷; however, we found no association between smoking and clinical outcomes among dMMR patients. This inconsistency with previous reports may reflect differences in study outcomes (ie, DFS v mortality), the distribution of stage, treatment, the duration of follow-up, and/or small numbers within patient subgroups ($n = 215$ dMMR patients in our analysis and $n = 255$ MSI-H patients in the prior analysis¹³). It is also plausible that the selection protocols and inclusion criteria for our randomized trial population differed from those of the prior population-based study in a manner that contributed to differences in associations; compared with the population-based study, the prevalence of ever smoking was lower in this study (53% v 59%), as was the prevalence of current smoking (7% v 18%). Overall, however, our findings and those of the previous analysis highlight the importance of considering patient and tumor characteristics in evaluating associations with potential prognostic factors for colon cancer.

Our findings are consistent with the substantial evidence of heterogeneity in the association between smoking and colon cancer risk according to various tumor markers.^{3,4,6-8,33,34} In particular, recent analyses from the Iowa Women's Health Study indicated that smoking is most strongly, if not exclusively, associated with an increased risk of *BRAF* mutated,³ MSI-H,³ and *KRAS* wild-type CRC.³³ Several other studies have also noted that smoking is more strongly associated with risk of MSI-H CRC than with risk of MSS disease.^{4,6,7} Consistent

Fig 3. Disease-free survival by cigarette smoking history among patients with (A) *KRAS* wild-type, (B) *KRAS* mutated, (C) *BRAF* wild-type, and (D) *BRAF* mutated colon cancer. M.HR, multivariate-adjusted hazard ratio.

with such heterogeneity, smokers in our study population were more likely than never-smokers to have *BRAF* mutated colon cancers and to have dMMR cancers. Such observations may relate to an effect of smoking on induction of methylation-related tumorigenesis: smoking has been associated with the induction of CpG island methylation,^{35,36} which is more frequently observed in *BRAF* mutated colon cancers³⁰ and is responsible for the dMMR phenotype in patients with nonfamilial disease.³⁷ However, we found that smoking was not associated with DFS among patients with these tumor characteristics, suggesting that the effect of smoking on risk of *BRAF* mutated and dMMR colon cancers is not mediated via an effect on tumor progression but rather via an effect on tumor initiation. In contrast, the fact that we found smoking to be associated with significantly shorter DFS in patients with colon cancer with *BRAF* wild-type, *KRAS* mutated, or pMMR tumors (ie, disease subtypes not previously associated with smoking in risk factor studies^{3,4,6-8}) suggests that smoking may have an impact on colon cancer progression and/or response to treatment through pathway-specific mechanisms that remain to be defined. The thousands of compounds contained in cigarette smoke could plausibly

have an impact on multiple pathways of tumor initiation, progression, and treatment-response via numerous distinct mechanisms.³⁸ As one example, it has been observed that nicotine induces increased proliferation and decreased apoptosis, which may be mediated via activation of the PI3K/AKT pathway.³⁹ This effect could plausibly be synergistically amplified in the presence of a *KRAS* mutation, given that *KRAS* also activates PI3K. Additional research is necessary to better understand the mechanisms through which specific compounds in cigarette smoke have an impact on colon cancer risk and survival.

Results from this analysis should be interpreted in the context of study limitations. Although the study population was large, patients numbers were modest within some strata defined by patient or tumor characteristics, which reduced our ability to evaluate associations with more detailed aspects of smoking history. In addition, the study questionnaire could not fully capture all aspects of smoking history, because use patterns can be complex.⁴⁰ Because information on smoking history was collected only at the time of study enrollment, we were unable to evaluate associations with postdiagnostic smoking or

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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