Aspirin Use and Risk of Colorectal Cancer According to *BRAF* Mutation Status

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Importance Aspirin use reduces the risk of colorectal carcinoma. Experimental evidence implicates a role of RAF kinases in up-regulation of prostaglandin-endoperoxide synthase 2 (PTGS2, cyclooxygenase 2), suggesting that *BRAF*-mutant colonic cells might be less sensitive to the antitumor effects of aspirin than *BRAF*–wild-type neoplastic cells.

Objective To examine whether the association of aspirin intake with colorectal cancer risk differs according to status of tumor *BRAF* oncogene mutation.

Design and Setting We collected biennial questionnaire data on aspirin use and followed up participants in the Nurses' Health Study (from 1980) and the Health Professionals Follow-up Study (from 1986) until July 1, 2006, for cancer incidence and until January 1, 2012, for cancer mortality. Duplication-method Cox proportional cause-specific hazards regression for competing risks data was used to compute hazard ratios (HRs) for colorectal carcinoma incidence according to *BRAF* mutation status.

Main Outcomes and Measures Incidence of colorectal cancer cases according to tumor *BRAF* mutation status.

Results Among 127 865 individuals, with 3 165 985 person-years of follow-up, we identified 1226 incident rectal and colon cancers with available molecular data. Compared with nonuse, regular aspirin use was associated with lower *BRAF*–wild-type cancer risk (multivariable HR, 0.73; 95% CI, 0.64 to 0.83; age-adjusted incidence rate difference [RD], −9.7; 95% CI, −12.6 to −6.7 per 100 000 person-years). This association was observed irrespective of status of tumor PTGS2 expression or *PIK3CA* or *KRAS* mutation. In contrast, regular aspirin use was not associated with a lower risk of *BRAF*-mutated cancer (multivariable HR, 1.03; 95% CI, 0.76 to 1.38; age-adjusted, incidence RD, 0.7; 95% CI, −0.3 to 1.7 per 100 000 person-years: *P* for heterogeneity = .037, between *BRAF*–wild-type vs *BRAF*-mutated cancer risks). Compared with no aspirin use, aspirin use of more than 14 tablets per week was associated with a lower risk of *BRAF*–wild-type cancer (multivariable HR, 0.43; 95% CI, 0.25 to 0.75; age-adjusted incidence RD, −19.8; 95% CI, −26.3 to −13.3 per 100 000 person-years). The relationship between the number of aspirin tablets per week and colorectal cancer risk differed significantly by *BRAF* mutation status (*P* for heterogeneity = .005).

Conclusions and Relevance Regular aspirin use was associated with lower risk of *BRAF*–wild-type colorectal cancer but not with *BRAF*-mutated cancer risk. These findings suggest that *BRAF*-mutant colon tumor cells may be less sensitive to the effect of aspirin. Given the modest absolute risk difference, further investigations are necessary to determine clinical implications of our findings.

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COLORECTAL CANCER IS A LEADING cause of cancer-related mortality worldwide. Randomized controlled trials have demonstrated that aspirin use reduces the risk of colorectal neoplasia,[1,2] including the risk of colorectal cancer in individuals with Lynch syndrome.[3] Aspirin is an inhibitor of prostaglandin-endoperoxide synthase 2 (PTGS2, alias cyclooxygenase 2), a key mediator of inflammatory responses.[4] We have previously shown that aspirin use is associated with a lower risk of colorectal cancer with PTGS2 overexpression.[5] However, since colorectal cancer represents a complex disease that cannot be explained by a single

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biomarker,[6] the association of aspirin with various tumorigenic processes requires further investigation, which may help develop effective preventive strategies.[7]

Colorectal cancers develop through accumulation of genetic and epigenetic alterations and through tumor-host interactions (including immune and inflammatory reactions) in the tumor microenvironment.[8,9] *BRAF* is a member of the RAF kinase family, and an important regulator of the mitogen-activated protein kinase (MAPK) pathway.[8-10] Activating mutations in the *BRAF* oncogene are observed in approximately 10% to 15% of colorectal cancers.[8,9] Experimental evidence suggests that RAF-MAPK signaling plays an important role in up-regulation of PTGS2 activity and prostaglandin E_2 synthesis.[11,12] Considering that oncogenic *BRAF* mutation causes constitutive activation of RAF-MAPK signaling, we hypothesized that *BRAF*-mutant colonic cells might be less sensitive to the antitumor effects of aspirin, whereas *BRAF*–wild-type neoplastic cells might be more susceptible to its antitumor effects.

To test this hypothesis, we examined the association of aspirin use with the risk of colorectal cancer according to *BRAF* mutation status within 2, large, US nationwide prospective cohort studies that provided detailed and updated information on aspirin use. Because of the close relationship among RAF, RAS, and phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K), we additionally examined the association between regular aspirin use and incident colorectal cancer according to *BRAF* mutation status in strata of PTGS2 expression, *PIK3CA* mutation, and *KRAS* mutation status. As an exploratory analysis, we examined patient survival according to postdiagnosis aspirin use and *BRAF* mutation status.

METHODS

Study Population

The Nurses' Health Study (NHS) was established in 1976 as a prospective cohort of 121 701 US registered female nurses who were aged 30 to 55 years at enrollment. The Health Professionals Follow-up Study (HPFS) was initiated in 1986 as a prospective cohort of 51 529 US male health professionals who were aged 40 to 75 years.[13] Biennial questionnaires were used to update data on lifestyle factors. Based on the self-report, demographic characteristics including ethnicity were assessed. Ninety-eight percent of the NHS and 95% of the HPFS participants were non-Hispanic white.

Informed consent was obtained from all participants. This study was approved by Human Subjects Committees at Harvard School of Public Health and Brigham and Women's Hospital.

Assessment of Aspirin Use

We have previously published a detailed description of the collection of information on aspirin use and the definition of regular aspirin use in these cohorts.[5] Briefly, in the NHS, aspirin use was first assessed in 1980 and every 2 years thereafter, except in 1986. The NHS participants were asked whether they took aspirin in most weeks, the number of tablets taken per week, and years of aspirin usage. We updated the information on the number of aspirin tablets taken per week (in categories) every 2 years. In the NHS, regular aspirin users were defined as women who reported consumption of 2 or more aspirin tablets per week and nonusers as women who used fewer than 2 tablets per week or no aspirin.

In the HPFS, in 1986 and every 2 years thereafter, participants were asked whether they used aspirin 2 or more times per week. Beginning in 1992, the mean number of tablets taken per week was assessed. In the HPFS, regular aspirin users were defined as men who reported consumption of aspirin at least 2 times per week and nonusers as men who consumed fewer than 2 times per week or no aspirin.

For both cohorts, participants were specifically asked about standard-dose (325 mg) aspirin tablets. Beginning in 1992, to reflect secular trends in aspirin use, participants were also asked to convert intake of 4 baby (81 mg) aspirin to 1 standard aspirin tablet in their responses. Aspirin dose was assessed using cumulative mean of tablets per week, which was the mean of all available data up to the start of each 2-year follow-up interval. We further evaluated duration of regular aspirin use (in years).[5] As previously described,[14] the major reasons for aspirin use among women were headache, arthritis and other musculoskeletal pain, and cardiovascular disease prevention. Among men, the major reasons were cardiovascular disease prevention, musculoskeletal pain, cardiovascular disease, and headache.

Assessment of Colorectal Cancer Cases

Incident colorectal cancer cases were ascertained by biennial questionnaire, the use of the National Death Index, and medical record review. Study physicians, unaware of exposure information, reviewed medical and pathological records to retrieve information on tumor location and disease stage. Considering the colorectal continuum model,[15,16] we combined rectal and colon cancers to maximize statistical power.

We collected available tumor specimens from pathology laboratories across the United States. As previously reported, the baseline characteristics of participants with colorectal cancer with available tissue molecular data were similar to those of participants without available molecular data.[17] A single pathologist (S.O.) reviewed tumor tissue slides and recorded pathological features.

BRAF, *KRAS*, *PIK3CA*, and Other Tumor Molecular Analyses

DNA was extracted from paraffin-embedded archival tumor tissue.[13] Polymerase chain reaction and Pyrosequencing were performed for *BRAF* (HGNC:1097; GenBank NM_004333) codon 600,[18] *KRAS* (HGNC:6407; GenBank NM_033360) codons 12 and 13,[19] and *PIK3CA* (HGNC:8975;

GenBank NM_006218) exons 9 and 20.[20] Microsatellite instability, CpG island methylator phenotype, and LINE-1 methylation, which were used in survival analysis models, were assessed as previously described.[21-24]

Immunohistochemistry for PTGS2 Expression

Prostaglandin-endoperoxide synthase 2 immunohistochemistry was performed using anti-PTGS2 (cyclooxygenase 2) antibody (Cayman Chemical; dilution 1:300), as previously described.[5] A single investigator (S.O.), unaware of other data, interpreted tumor PTGS2 expression level (absent, weak, moderate, or strong), compared with adjacent normal colonic epithelium. A random sample of 124 cancers was examined by a second investigator (T.M.), and concordance between the 2 observers was 0.85 ($\kappa = 0.69$, $P < .001$).

Statistical Analysis

A detailed description of the statistical analysis, including our analysis of cancer mortality, is provided in the eSupplement (available at http://www.jama.com). We used SAS software version 9.2 (SAS Institute Inc) for all statistical analyses. All *P* values were 2-sided and a *P* value $<.05$ was considered statistically significant. We included participants who provided baseline data on aspirin use in 1980 for the NHS and in 1986 for the HPFS. We excluded participants with a history of cancer (except for nonmelanoma skin cancer), inflammatory bowel disease, or familial polyposis at baseline. We followed up participants from the date of return of the baseline questionnaire, through July 1, 2006, for cancer incidence analysis, and through January 1, 2012, for cancer mortality analysis. Participants who died from causes other than colorectal cancer were censored.

To examine differential associations of aspirin use with colorectal cancer risk by tumor molecular subtype, we used Cox proportional cause-specific hazards regression model with a duplication method for competing risks data. This method permits estimation of separate associations of a risk factor (ie, aspirin use) with each tumor subtype, and has been used to assess whether a risk factor has statistically different regression coefficients for different tumor subtypes.[5,25]

In incidence analysis of one subtype, incidence of the other tumor subtype or tumor of unknown subtype was treated as censored data. A test of heterogeneity was conducted using a likelihood ratio test that compared the model that allowed for different associations of aspirin use according to tumor subtype, with a model that assumed a common association. Trend tests across categories of aspirin dose, and duration of regular use, were performed by assigning median values for these categories and treating the variables as continuous terms in the model. All analyses were stratified by age (in months), sex (in the combined cohort analysis), and calendar year of the questionnaire cycle.

Multivariable HRs were further adjusted for body mass index, family history of colorectal cancer in any first-degree relative, smoking status, lower endoscopy status, postmenopausal hormone use (for women only), history of diabetes, history of cardiovascular disease, physical activity, red meat intake, alcohol consumption, total caloric intake, folate intake, calcium intake, and current multivitamin use. Because information on other relevant medications (cholesterol-lowering drugs, antihypertensive drugs, and nonsteroidal anti-inflammatory drugs) was comprehensively collected beginning in 1990 onward in the NHS, we conducted a sensitivity analysis using data from 1990 for the NHS, and from 1986 for the HPFS to include these factors in our multivariable model. We used the most updated available information for all variables prior to each 2-year follow-up period, and modeled all variables as time-varying variables to take into account potential changes over follow-up time. If participants missed aspirin or other covariates information in biennial questionnaires, we used most recent available information from the past questionnaires.

RESULTS

Aspirin Use and Colorectal Cancer Risk by *BRAF* Status

At the baseline, there were 82 095 women in the NHS in 1980 and 45 770 men in the HPFS in 1986. TABLE 1 shows the demographic characteristics of the participants in 1994 according to regular aspirin use status. During 28 years and 3 165 985 person-years of follow-up, we documented 1226 incident cases of colorectal cancer (41% of all colorectal cancer cases) with available tissue molecular data. As previously reported,[5] both women and men who used aspirin regularly had a significantly lower overall risk of colorectal cancer compared with nonusers (TABLE 2). Multivariable-adjusted models yielded similar risk estimates to age-adjusted models.

For *BRAF*–wild-type cancer, age-adjusted incidence rates per 100 000 person-years were 40.2 (95% CI, 38.4 to 42.0) among nonusers and 30.5 (95% CI, 28.2 to 32.9) among regular aspirin users. Regular aspirin use was associated with a significantly lower risk of *BRAF*–wild-type cancer (multivariable HR, 0.73; 95% CI, 0.64 to 0.83; age-adjusted incidence rate difference [RD], −9.7; 95% CI, −12.6 to −6.7 per 100 000 person-years). For *BRAF*-mutated cancer, age-adjusted incidence rates per 100 000 person-years were 5.0 (95% CI, 4.4 to 5.6) among nonusers and 5.7 (95% CI, 4.9 to 6.5) among regular aspirin users. Regular aspirin use was not associated with a lower risk of *BRAF*-mutated cancer (multivariable HR, 1.03; 95% CI, 0.76 to 1.38; age-adjusted incidence RD, 0.7; 95% CI, −0.3 to 1.7 per 100 000 person-years). The association of aspirin use with colorectal cancer risk differed significantly according to *BRAF* mutation status (*P* for heterogeneity = .037). In a sensitivity analysis that included use of cholesterol-lowering drugs, antihypertensive drugs, and nonsteroidal anti-inflammatory drugs in the multivari-

able model, we found that inclusion of the medication data in the model did not substantially alter the results (eTable 1, available at http//:www.jama.com).

We observed a lower risk of *BRAF*–wild-type cancer with increasing aspirin tablets per week (P for trend<.001); however, we did not observe a significant trend in risk reduction for *BRAF*-mutated cancer (P for trend=.62; TABLE 3). The association of aspirin tablets per week with cancer risk differed significantly by *BRAF* mutation status (P for heterogeneity=.005). Compared with individuals who reported no aspirin use (age-adjusted incidence rate, 36.6; 95% CI, 34.4 to 38.7 per 100 000 person-years), a significantly lower risk of *BRAF*–wild-type cancer was observed among individuals who used 6 to 14 tablets of aspirin per week (age-adjusted incidence rates, 26.8; 95% CI, 24.1 to 29.4 per 100 000 person-years; multivariable HR, 0.70; 95% CI, 0.55 to 0.88; age-adjusted incidence RD, −9.8; 95% CI, −13.2 to −6.4 per 100 000 person-years) and among those who used more than 14 tablets of aspirin per week (age-adjusted incidence rate, 16.8; 95% CI, 10.7 to 22.9 per 100 000 person-years; multivariable HR, 0.43; 95% CI, 0.25 to 0.75; age-adjusted incidence RD, −19.8; 95% CI, −26.3 to −13.3 per 100 000 person-years).

We further examined the association between duration of regular aspirin use and colorectal cancer risk by *BRAF* mutation status (TABLE 4). Longer duration of aspirin use was associated with significant risk reduction for *BRAF*–wild-type cancer (P for trend <.001), whereas duration of aspirin use was not significantly associated with *BRAF*-mutated cancer risk (P for trend=.37). However, a formal test for heterogeneity of the association according to *BRAF* mutation status did not reach statistical significance (P for heterogeneity=.17).

Table 1. Age-Adjusted Demographic Characteristics According to Regular Aspirin Use Status in 1994[a]

	Nurses' Health Study		Health Professionals Follow-up Study		Total	
	Nonusers (n = 50 771)	Regular Users (n = 32 692)	Nonusers (n = 23 817)	Regular Users (n = 19 498)	Nonusers (n = 74 588)	Regular Users (n = 52 190)
Age, mean (SD), y[b]	59.8 (7.1)	61.6 (7.0)	60.2 (9.4)	62.8 (9.4)	60.0 (7.9)	62.0 (8.0)
BMI, mean (SD)	24.8 (4.2)	25.6 (4.7)	25.6 (3.4)	25.9 (3.3)	25.1 (4.0)	25.7 (4.2)
Family history of colorectal cancer in any first-degree relative, %	13	13	10	10	12	12
Smoking status, %						
Never	45	43	48	43	46	43
Former	41	43	44	50	42	46
Current	14	14	8	7	12	12
Lower endoscopy status, %						
No endoscopy	65	62	50	46	59	55
History of adenomatous polyps	2	2	3	3	2	2
Negative endoscopy[c]	33	36	47	52	38	43
History of diabetes, %	6	8	5	6	6	7
History of cardiovascular disease, %	6	13	8	22	7	16
Postmenopausal hormone use, ever, %	64	70				
Physical activity and diet, mean (SD)						
Physical activity, METs h/wk[d]	17.2 (17.9)	16.6 (17.4)	30.6 (29.1)	31.4 (28.7)	21.6 (23.1)	22.3 (23.5)
Red meat intake, servings/d	1.0 (0.5)	1.1 (0.5)	1.2 (0.9)	1.2 (0.9)	1.1 (0.7)	1.1 (0.7)
Alcohol consumption, g/d	5.7 (8.8)	6.2 (9.2)	10.2 (13.6)	11.5 (14.0)	7.1 (10.8)	8.2 (11.5)
Total calories, kcal/d	1684 (425)	1728 (429)	1962 (554)	1983 (549)	1773 (488)	1823 (493)
Folate intake, μg/d	395 (187)	416 (177)	484 (233)	520 (233)	423 (207)	455 (206)
Calcium intake, mg/d	907 (329)	950 (338)	893 (358)	918 (353)	903 (338)	938 (344)
Multivitamin use, %	44	51	43	54	43	52
Medication use, %						
Cholesterol-lowering drug use	6	9	4	11	5	10
Antihypertensive drug use	23	35	17	29	21	33
Nonsteroidal anti-inflammatory drug use	18	32	10	13	16	25

Abbreviations: BMI, body mass index, calculated as weight in kilograms divided by height in meters squared; MET, metabolic equivalent task.

[a] In the Nurses' Health Study, regular aspirin use was defined as the consumption of at least two 325-mg tablets per week and nonuse was defined as consumption of fewer than 2 tablets per week. In the Health Professionals Follow-up Study, regular aspirin use was defined as the consumption of aspirin at least 2 times per week and nonuse use was defined as the consumption of aspirin fewer than 2 times per week. Values are standardized to the age distribution of the study population.

[b] Value is not age adjusted.

[c] Endoscopy without detection of adenomatous polyps or cancer.

[d] MET is calculated according to the frequency of a range of physical activities in 1986 for both women and men.

Aspirin and Cancer Risk According to Combined Tumor Subtype

In an earlier study using these cohorts,[5] we demonstrated that regular aspirin use was associated with a lower risk of PTGS2-positive cancer but not with PTGS2-negative cancer risk. We evaluated the association between aspirin use and *BRAF*–wild-type cancer risk by strata of tumor PTGS2 expression (TABLE 5). Regular aspirin use was associated with a significantly lower risk of *BRAF*–wild-type PTGS2-positive cancer (multivariable HR, 0.67; 95% CI, 0.56 to 0.81; age-adjusted incidence RD, −7.2; 95% CI, −9.7 to −4.6 per 100 000 person-years). These data suggest that the association between aspirin use and a lower risk of *BRAF*–wild-type cancer is primarily confined to tumors positive for PTGS2.

In the analysis of combined *BRAF-PIK3CA* mutation status, regular aspirin use appeared to be associated with

Table 2. Regular Use of Aspirin and Incident Colorectal Cancer by *BRAF* Mutation Status[a]

	Hazard Ratio (95% CI)						
	Nurses' Health Study		Health Professionals Follow-up Study		Total		
	Nonusers	Regular Users	Nonusers	Regular Users	Nonusers	Regular Users	*P* for Heterogeneity[b]
Person-years	1 364 173	907 622	484 644	409 545	1 848 818	1 317 167	
All colorectal cancer							
No. of incidences	440	245	294	247	734	492	
Age-adjusted	1 [Referent]	0.73 (0.63-0.86)	1 [Referent]	0.82 (0.69-0.97)	1 [Referent]	0.77 (0.68-0.86)	
Multivariable[c]	1 [Referent]	0.72 (0.61-0.84)	1 [Referent]	0.86 (0.72-1.03)	1 [Referent]	0.77 (0.68-0.87)	
BRAF–wild-type cancer							
No. of incidences	364	183	274	223	638	406	
Age-adjusted	1 [Referent]	0.67 (0.56-0.81)	1 [Referent]	0.79 (0.66-0.95)	1 [Referent]	0.73 (0.64-0.83)	
Multivariable[c]	1 [Referent]	0.66 (0.55-0.79)	1 [Referent]	0.84 (0.69-1.01)	1 [Referent]	0.73 (0.64-0.83)	
BRAF-mutated cancer							.037
No. of incidences	76	62	20	24	96	86	
Age-adjusted	1 [Referent]	0.99 (0.71-1.39)	1 [Referent]	1.14 (0.62-2.09)	1 [Referent]	1.03 (0.77-1.38)	
Multivariable[c]	1 [Referent]	0.98 (0.70-1.38)	1 [Referent]	1.18 (0.64-2.16)	1 [Referent]	1.03 (0.76-1.38)	

[a] In the Nurses' Health Study, regular aspirin use was defined as the consumption of at least two 325-mg tablets per week and nonuse was defined as consumption of fewer than 2 tablets per week. In the Health Professionals Follow-up Study, regular aspirin use was defined as the consumption of aspirin at least 2 times per week and nonuse was defined as the consumption of aspirin fewer than 2 times per week.
[b] *P* for heterogeneity test for the heterogeneity of the association of regular aspirin use with colorectal cancer defined by molecular subtype in the multivariable models.
[c] The multivariable hazard ratio was further adjusted for body mass index (<25 vs 25-30 vs ≥30; calculated as weight in kilograms divided by height in meters squared), smoking status (never vs former vs current), family history of colorectal cancer in any first-degree relative, endoscopy status (no endoscopy vs history of adenomatous polyps vs negative endoscopy), history of diabetes, history of cardiovascular disease, physical activity level (quintiles of mean metabolic equivalent task-hours per week), red meat intake (quintiles of servings per day), total calorie intake (quintiles of kcal/d), alcohol consumption (0 or quartiles of g/d), folate intake (quintiles of μg/d), calcium intake (quintiles of mg/d), and current multivitamin use. Models were adjusted for postmenopausal hormone use in the analyses of women.

Table 3. Aspirin Tablets per Week and Incident Colorectal Cancer by *BRAF* Mutation Status[a]

	Hazard Ratio (95% CI) by No. of Aspirin Tablets Used per Week						
	0	0.5-1.5	2-5	6-14	>14	*P* for Trend[b]	*P* for Heterogeneity[c]
Person-years	607 248	1 055 816	632 189	441 965	112 975		
All colorectal cancer							
No. of incidences	207	447	247	165	22		
Age adjusted	1 [Referent]	1.03 (0.87-1.22)	0.83 (0.69-1.01)	0.74 (0.60-0.91)	0.56 (0.36-0.87)	<.001	
Multivariable[d]	1 [Referent]	1.06 (0.89-1.26)	0.86 (0.71-1.04)	0.74 (0.60-0.92)	0.56 (0.36-0.88)	<.001	
BRAF–wild-type cancer							
No. of incidences	178	377	213	130	14		
Age- adjusted	1 [Referent]	1.05 (0.87-1.27)	0.86 (0.70-1.05)	0.69 (0.55-0.87)	0.43 (0.25-0.75)	<.001	
Multivariable[d]	1 [Referent]	1.08 (0.90-1.31)	0.88 (0.72-1.08)	0.70 (0.55-0.88)	0.43 (0.25-0.75)	<.001	
BRAF-mutated cancer							.005
No. of incidences	29	70	34	35	8		
Age adjusted	1 [Referent]	0.92 (0.59-1.42)	0.70 (0.42-1.16)	0.96 (0.59-1.58)	1.13 (0.51-2.50)	.63	
Multivariable[d]	1 [Referent]	0.97 (0.62-1.51)	0.73 (0.44-1.21)	0.99 (0.60-1.63)	1.20 (0.54-2.64)	.62	

[a] Standard-dose (325 mg)–equivalent tablets per week. Analysis in the Health Professionals Follow-up Study was confined to follow-up after 1992, the baseline questionnaire in which we began to routinely collect information on aspirin tablets per week.
[b] Based on the linear trend test by using the median value of each category.
[c] *P* for heterogeneity tests for the heterogeneity of the linear association of aspirin dose with colorectal cancer defined by molecular subtype in the multivariable models.
[d] The multivariable hazard ratio was further adjusted for body mass index (<25 vs 25-30 vs ≥30, calculated as weight in kilograms divided by height in meters squared), smoking status (never vs former vs current), family history of colorectal cancer in any first-degree relative, endoscopy status (no endoscopy vs history of adenomatous polyps vs negative endoscopy), history of diabetes, history of cardiovascular disease, physical activity level (quintiles of mean metabolic equivalent task-hours per week), red meat intake (quintiles of servings per day), total calorie intake (quintiles of kcal/d), alcohol consumption (0 or quartiles of g/d), folate intake (quintiles of μg/d), calcium intake (quintiles of mg/d), and current multivitamin use.

Table 4. Duration of Regular Aspirin Use and Incident Colorectal Cancer by *BRAF* Mutation Status[a]

	Hazard Ratio (95% CI) by Years of Regular Aspirin Use				*P* for Trend[b]	*P* for Heterogeneity[c]
	0	1-5	6-10	>10		
Person-years	1 162 522	644 890	550 075	808 498		
All colorectal cancer						
No. of incidences	408	273	277	268		
Age adjusted	1 [Referent]	1.03 (0.88-1.20)	0.94 (0.81-1.10)	0.69 (0.59-0.81)	<.001	
Multivariable[d]	1 [Referent]	1.04 (0.88-1.21)	0.96 (0.82-1.13)	0.69 (0.58-0.81)	<.001	
BRAF–wild-type cancer						
No. of incidences	352	234	242	216		
Age adjusted	1 [Referent]	1.03 (0.87-1.22)	0.97 (0.82-1.15)	0.67 (0.56-0.80)	<.001	
Multivariable[d]	1 [Referent]	1.03 (0.87-1.23)	0.98 (0.83-1.17)	0.66 (0.55-0.80)	<.001	
BRAF-mutated cancer						.17
No. of incidences	56	39	35	52		
Age adjusted	1 [Referent]	1.01 (0.67-1.52)	0.79 (0.52-1.22)	0.81 (0.55-1.20)	.36	
Multivariable[d]	1 [Referent]	1.04 (0.69-1.57)	0.82 (0.53-1.26)	0.82 (0.55-1.21)	.37	

[a] In the Nurses' Health Study, regular aspirin use was defined as the consumption of at least two 325-mg tablets per week. In the Health Professionals Follow-up Study, regular aspirin use was defined as the consumption of aspirin at least 2 times per week.
[b] Based on the linear trend test by using the median value of each category.
[c] *P* for heterogeneity tests for the heterogeneity of the linear association of aspirin duration with colorectal cancer defined by molecular subtype in the multivariable models.
[d] The multivariable hazard ratio was further adjusted for body mass index (<25 vs 25-30 vs ≥30, calculated as weight in kilograms divided by height in meters squared), smoking status (never vs former vs current), family history of colorectal cancer in any first-degree relative, endoscopy status (no endoscopy vs history of adenomatous polyps vs negative endoscopy), history of diabetes, history of cardiovascular disease, physical activity level (quintiles of mean metabolic equivalent task-hours per week), red meat intake (quintiles of servings per day), total calorie intake (quintiles of kcal/d), alcohol consumption (0 or quartiles of g/d), folate intake (quintiles of μg/d), calcium intake (quintiles of mg/d), and current multivitamin use.

a lower risk of *BRAF*–wild-type cancer, regardless of *PIK3CA* mutation status (eTable 2). Moreover, the association between regular aspirin use and a lower risk of *BRAF*–wild-type cancer appeared to be independent of *KRAS* mutation status (eTable 3).

Postdiagnosis Aspirin Use and Patient Survival According to *BRAF* Status

We did not observe significant interaction between postdiagnosis aspirin use and *BRAF* mutation status in cancer-specific or overall survival analysis (eTable 4). Further analysis of survival among patients with colorectal cancer according to postdiagnosis aspirin use and combined *BRAF* and *PIK3CA* mutation status had limited statistical power (eTable 4).

DISCUSSION

In 2 large prospective cohort studies, we found that regular aspirin use was associated with a lower risk of *BRAF*–wild-type colorectal cancer, but not with *BRAF*-mutated cancer risk. The lower *BRAF*–wild-type cancer risk was more pronounced with increasing aspirin tablets per week. Furthermore, the association of aspirin use with lower cancer risk appeared to be most evident for *BRAF*–wild-type PTGS2-positive cancer, whereas aspirin use was not associated with *BRAF*-mutated cancer regardless of tumor PTGS2 expression status. These findings support the hypothesis that *BRAF*-mutated cells may show resistance to the anticancer effects of aspirin due to up-regulation of the MAPK pathway. Previous experimental studies have shown that activating *BRAF* mutation results in MAPK-mediated up-regulation of PTGS2, and prostaglandin E2 production.[11,12,26] Considering that *BRAF* mutation might constitutively up-regulate PTGS2 activity, we speculate that, within *BRAF*-mutant neoplastic cells, PTGS2 may be persistently active even with low expression level, potentially conferring resistance to the effect of aspirin. In contrast, within *BRAF*–wild-type cells, PTGS2 activity may be relatively inducible and overexpression of PTGS2 may function as a marker of a tumor cell that may be more susceptible to the effects of aspirin. The exact mechanisms underlying the interplay of aspirin, PTGS2, and *BRAF* mutation need to be elucidated by further investigations.

There was no statistically significant interaction between postdiagnosis aspirin use and *BRAF* mutation status in colorectal cancer-specific or overall survival analysis. This suggests that the potential protective effect of aspirin may differ by *BRAF* status in the early phase of tumor evolution before clinical detection but not during later phases of tumor progression. One reason for these seemingly discrepant findings in cancer incidence analysis compared with cancer survival analysis may be related to differences in the effect of aspirin according to tumor microenvironmental changes. During tumor evolution, colonic cells encounter multifactorial molecular events, including changes in genome, epigenome, proteome, metabolome, and interactome. Thus, the interactive effect of aspirin use and tumor molecular characteristics might vary as a tumor's microenvironment evolves.

The association between regular aspirin use and a lower risk of *BRAF*–wild-type cancer appeared independent of *PIK3CA* and *KRAS* mutation status. Together with our previous data,[13] the interplay between aspirin and *PIK3CA* mutation status may be opera-

tive in later phases rather than earlier phases of tumor evolution.

The identification of specific cancer subtypes that are prevented by aspirin is important for several reasons.[7,27] First, it enhances our understanding of the molecular pathogenesis of colorectal neoplasia and the mechanisms through which aspirin may exert its antineoplastic effects. Second, development of clinical, genetic, or molecular predictors of specific subtypes of colorectal cancer might lead to the development of more tailored screening or chemopreventive strategies. Nevertheless, given the modest absolute risk difference, further investigations are necessary to evaluate clinical implications of our findings. Lastly, our data provide additional support for a causal association between aspirin use and risk reduction for a specific subtype of colorectal cancers. Accumulating evidence supports preventive effect of aspirin against colorectal cancer.[1-3,28-30] The findings of clinical studies in Lynch syndrome mutation carriers further support our results because the vast majority of cancers associated with Lynch syndrome are *BRAF*–wild-type.[31]

Several attributes of the NHS and the HPFS cohorts strengthen our study and its findings. First, because updated information on aspirin use was prospectively collected over 28 years, we were able to assess the long-term association of aspirin exposure with colorectal cancer, which can take many years to evolve. Second, our detailed, updated exposure data allowed us to control for the effects of potential confounding by other dietary and lifestyle factors implicated in colorectal carcinogenesis. Third, our present study exploits a molecular pathological epidemiology analytic approach,[8,32] which has enabled us to elucidate the association between a specific exposure and molecular subtype of cancer, to provide better insight into disease pathogenesis.[13,33-40]

Our study has limitations. The possibility of residual confounding by measured or unmeasured factors cannot be

Table 5. Regular Use of Aspirin and Incident Colorectal Cancer by PTGS2 Status and Combination of *BRAF*-PTGS2 Status[a]

	Hazard Ratio (95% CI)		
	Nonusers	Regular Users	*P* for Heterogeneity[b]
Person-years	1 848 818	1 317 167	
PTGS2 status			
PTGS2-negative cancer			
No. of incidences	211	170	
Age-adjusted	1 [Referent]	0.96 (0.79-1.18)	
Multivariable[c]	1 [Referent]	0.96 (0.78-1.18)	
PTGS2-positive cancer			.013
No. of incidences	419	234	
Age-adjusted	1 [Referent]	0.69 (0.59-0.81)	
Multivariable[c]	1 [Referent]	0.69 (0.59-0.82)	
BRAF-PTGS2 status			
BRAF–wild-type PTGS2-negative cancer			
No. of incidences	166	119	
Age-adjusted	1 [Referent]	0.85 (0.67-1.08)	
Multivariable[c]	1 [Referent]	0.86 (0.67-1.09)	
BRAF–wild-type PTGS2-positive cancer			
No. of incidences	355	191	
Age-adjusted	1 [Referent]	0.67 (0.56-0.80)	
Multivariable[c]	1 [Referent]	0.67 (0.56-0.81)	
BRAF-mutated PTGS2-negative cancer			.018
No. of incidences	37	38	
Age-adjusted	1 [Referent]	1.22 (0.77-1.93)	
Multivariable[c]	1 [Referent]	1.23 (0.78-1.94)	
BRAF-mutated PTGS2-positive cancer			
No. of incidences	34	34	
Age-adjusted	1 [Referent]	1.21 (0.75-1.95)	
Multivariable[c]	1 [Referent]	1.20 (0.74-1.94)	

Abbreviation: PTGS2, prostaglandin-endoperoxide synthase 2 (cyclooxygenase 2).

[a] In the Nurses' Health Study, regular aspirin use was defined as the consumption of at least two 325-mg tablets per week and nonuse was defined as consumption of fewer than 2 tablets per week. In the Health Professionals Follow-up Study, regular aspirin use was defined as the consumption of aspirin at least 2 times per week and nonuse was defined as the consumption of aspirin fewer than 2 times per week.

[b] *P* for heterogeneity tests for the heterogeneity of the association of regular aspirin use with colorectal cancer defined by molecular subtype (ie, the association for at least 1 subtype is significantly different from the association for at least 1 of the others) in the multivariable models.

[c] The multivariable hazard ratio was further adjusted for body mass index (<25 vs 25-30 vs ≥30, calculated as weight in kilograms divided by height in meters squared), smoking status (never vs former vs current), family history of colorectal cancer in any first-degree relative, endoscopy status (no endoscopy vs history of adenomatous polyps vs negative endoscopy), history of diabetes, history of cardiovascular disease, physical activity level (quintiles of mean metabolic equivalent task-hours per week), red meat intake (quintiles of servings per day), total calorie intake (quintiles of kcal/d), alcohol consumption (0 or quartiles of g/d), folate intake (quintiles of μg/d), calcium intake (quintiles of mg/d), and current multivitamin use.

excluded. Although colorectal cancer case ascertainment was well established in our cohorts, we were not able to retrieve tissue specimens from all incident cancers. Statistical power was limited, especially in the analysis of the number of aspirin tablets per week, due to *BRAF* mutations present in only approximately 10% to 15% of colorectal cancers.[10,15,41-43] The vast majority of participants were non-Hispanic white, and our findings may not be generalizable to other ethnic groups. Although our current study represented a hypothesis-driven analysis, we are aware of the various caveats associated with molecular pathological epidemiology and tumor subtype analyses.[8,32] Our results must be validated by independent studies, and further investigations are necessary to confirm the association of aspirin use with a lower risk of *BRAF*–wild-type cancer independent of other tumor markers.

In summary, regular aspirin use was associated with lower risk of *BRAF*–wild-type colorectal cancer but not with *BRAF*-mutated cancer risk. Nevertheless, given the modest absolute risk difference, further investigations are necessary to determine clinical implications of our findings.

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