ORIGINAL ARTICLE

Aspirin Use, Tumor *PIK3CA* Mutation, and Colorectal-Cancer Survival

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ABSTRACT

BACKGROUND

Regular use of aspirin after a diagnosis of colon cancer has been associated with a superior clinical outcome. Experimental evidence suggests that inhibition of prostaglandin-endoperoxide synthase 2 (PTGS2) (also known as cyclooxygenase-2) by aspirin down-regulates phosphatidylinositol 3-kinase (PI3K) signaling activity. We hypothesized that the effect of aspirin on survival and prognosis in patients with cancers characterized by mutated *PIK3CA* (the phosphatidylinositol-4,5-bisphosphonate 3-kinase, catalytic subunit alpha polypeptide gene) might differ from the effect among those with wild-type *PIK3CA* cancers.

METHODS

We obtained data on 964 patients with rectal or colon cancer from the Nurses' Health Study and the Health Professionals Follow-up Study, including data on aspirin use after diagnosis and the presence or absence of *PIK3CA* mutation. We used a Cox proportional-hazards model to compute the multivariate hazard ratio for death. We examined tumor markers, including PTGS2, phosphorylated AKT, *KRAS*, *BRAF*, microsatellite instability, CpG island methylator phenotype, and methylation of long interspersed nucleotide element 1.

RESULTS

Among patients with mutated-*PIK3CA* colorectal cancers, regular use of aspirin after diagnosis was associated with superior colorectal cancer–specific survival (multivariate hazard ratio for cancer-related death, 0.18; 95% confidence interval [CI], 0.06 to 0.61; P<0.001 by the log-rank test) and overall survival (multivariate hazard ratio for death from any cause, 0.54; 95% CI, 0.31 to 0.94; P=0.01 by the log-rank test). In contrast, among patients with wild-type *PIK3CA*, regular use of aspirin after diagnosis was not associated with colorectal cancer–specific survival (multivariate hazard ratio, 0.96; 95% CI, 0.69 to 1.32; P=0.76 by the log-rank test; P=0.009 for interaction between aspirin and *PIK3CA* variables) or overall survival (multivariate hazard ratio, 0.94; 95% CI, 0.75 to 1.17; P=0.96 by the log-rank test; P=0.07 for interaction).

CONCLUSIONS

Regular use of aspirin after diagnosis was associated with longer survival among patients with mutated-*PIK3CA* colorectal cancer, but not among patients with wild-type *PIK3CA* cancer. The findings from this molecular pathological epidemiology study suggest that the *PIK3CA* mutation in colorectal cancer may serve as a predictive molecular biomarker for adjuvant aspirin therapy. (Funded by The National Institutes of Health and others.)

Numerous observational and randomized, controlled studies have suggested a protective effect of regular use of aspirin on colorectal neoplasias.[1-7] The favorable outcome that has been associated with aspirin use after colorectal cancer is diagnosed[8-10] suggests that aspirin is a promising agent for adjuvant therapy. Accumulating data suggest that colorectal cancers are a heterogeneous group of diseases that potentially have differential responses to treatment.[11] The effect of postdiagnosis aspirin use on survival appears to differ according to tumor expression of PTGS2 (HGNC:9605, the official symbol for prostaglandin-endoperoxide synthase 2, also known as cyclooxygenase-2) as assessed by immunohistochemical techniques.[8] Considering the challenges in standardizing PTGS2 immunohistochemical assays across pathology laboratories, other molecular biomarkers — particularly those that can be assessed through a more standardized approach than immunohistochemistry — are needed to better identify patients with colorectal cancer who will derive a benefit from aspirin.

The phosphatidylinositol 3-kinase (PI3K) signaling pathway plays an important role in carcinogenesis.[12] Mutations in *PIK3CA* (the gene encoding phosphatidylinositol-4,5-bisphosphonate 3-kinase, catalytic subunit alpha polypeptide) are present in approximately 15 to 20% of colorectal cancers.[13-19] Up-regulation of PI3K enhances PTGS2 activity and prostaglandin E_2 synthesis, resulting in inhibition of apoptosis in colon-cancer cells.[20] Aspirin may suppress cancer-cell growth and induce apoptosis by blocking the PI3K pathway.[21] We therefore hypothesized that the effect of aspirin on survival among patients with mutated-*PIK3CA* colorectal cancers might differ from the effect among those with wild-type *PIK3CA* tumors. To test this hypothesis, we used two U.S. nationwide prospective cohort studies with data on aspirin use, tumor molecular characteristics, and patient outcomes.

METHODS

STUDY DESIGN

We used data from two prospective cohort studies, the Nurses' Health Study (NHS, involving 121,700 women who were enrolled in 1976) and the Health Professionals Follow-up Study (HPFS, involving 51,500 men who were enrolled in 1986).[22,23] Every 2 years, participants were sent follow-up questionnaires to update information on lifestyle factors and to identify newly diagnosed cancers and other diseases. The National Death Index was used to ascertain deaths of study participants. The cause of death was assigned by study physicians. Paraffin-embedded tissue blocks were collected from hospitals where participants with colorectal cancer had undergone colorectal resection or endoscopic biopsy (for preoperatively treated rectal cancer). Tumor-tissue data, information on aspirin use, and survival data were available for 1097 patients with colorectal cancer that was diagnosed before July 1, 2006. Among these patients, we used data from 964 patients for whom information about the presence or absence of *PIK3CA* mutation, based on analysis of tumor tissue, was available. Patients were followed until death or January 2011, whichever came first.

Written informed consent was obtained from all study participants. Tissue collection and analyses were approved by the human subjects committees at the Harvard School of Public Health and Brigham and Women's Hospital. The last two authors were responsible for the study concept and design. All authors acquired, analyzed, and interpreted the data. The first two authors and the last two authors take responsibility for the integrity of the data and the accuracy of the data analysis and vouch for the fidelity of the study to the protocol.

ASSESSMENT OF ASPIRIN USE

Assessment of aspirin use in the NHS and HPFS cohorts has been described in detail previously.[8] In 1980, NHS participants were asked whether they regularly used aspirin in most weeks, as well as the dose and duration of use; thereafter, this information was updated biennially (except in 1986). Beginning in 1986, HPFS participants were asked whether they regularly used aspirin two or more times each week; after 1992, information on the average dose used each week was requested. In both cohorts, the use of standard-dose (325-mg) aspirin tablets was documented. After 1992, to reflect the increasing use of low-dose (81-mg) aspirin (baby aspirin), participants were asked to convert four low-dose tablets to one standard-dose tablet in their response. The reasons for aspirin use were documented, as described previously, and included headache, arthritis, and other musculoskeletal pain, as well as cardiovascular disease and its pre-

vention.[8] As described previously,[8] aspirin use was defined as regular use of aspirin during most weeks, whereas nonuse of aspirin was defined as no regular use of aspirin during most weeks.

ANALYSES OF *PIK3CA*, *BRAF*, *KRAS*, MICROSATELLITE INSTABILITY, AND DNA METHYLATION

DNA was extracted from paraffin-embedded tissues.[15] Polymerase chain reaction and pyrosequencing of *PIK3CA* (exons 9 and 20),[15,24] *KRAS* (codons 12 and 13),[25] and *BRAF* (codon 600)[26] were performed as previously described. Microsatellite instability status was determined as previously described.[27] Methylation analyses of long interspersed nucleotide element 1 (LINE-1) elements[28,29] and of eight CpG island methylator phenotype–specific loci[27] (*CACNA1G*, *CDKN2A*, *CRABP1*, *IGF2 MLH1*, *NEUROG1*, *RUNX3*, and *SOCS1* with the use of the MethyLight technique[30,31]) were performed as previously described.

PTGS2 AND PHOSPHORYLATED AKT IMMUNOHISTOCHEMISTRY

Immunohistochemical analyses to detect PTGS2 and phosphorylated AKT (phospho-AKT) were performed as previously described,[22,32] and details of the methods are described in the Supplementary Appendix, available with the full text of this article at NEJM.org.

Table 1. Baseline Characteristics of Patients with Colorectal Cancer, According to *PIK3CA* Mutation Status and Regular Use or Nonuse of Aspirin after Diagnosis.*

Characteristic	All Patients (N=964)	Wild-Type *PIK3CA*		Mutant *PIK3CA*		P Value
		No Aspirin Use (N=466)	Aspirin Use (N=337)	No Aspirin Use (N=95)	Aspirin Use (N=66)	
Sex — no. (%)†						0.30
Male	429 (44)	195 (42)	155 (46)	44 (46)	35 (53)	
Female	535 (56)	271 (58)	182 (54)	51 (54)	31 (47)	
Age — yr	68.0±8.6	67.5±8.4	69.1±8.5	69.1±8.0	69.1±10.1	0.12
Year of diagnosis — no. (%)						0.06
Before 1997	422 (44)	224 (48)	134 (40)	40 (42)	24 (36)	
1997 or later	542 (56)	242 (52)	203 (60)	55 (58)	42 (64)	
History of colorectal cancer in first-degree relative — no. (%)						0.38
No	777 (81)	386 (83)	266 (79)	73 (77)	52 (79)	
Yes	187 (19)	80 (17)	71 (21)	22 (23)	14 (21)	
Body-mass index — no./total no. (%)‡						0.99
<30	788/963 (82)	380/466 (82)	276/337 (82)	77/94 (82)	55/66 (83)	
≥30	175/963 (18)	86/466 (18)	61/337 (18)	17/94 (18)	11/66 (17)	
Aspirin use before diagnosis — no. (%)						<0.001
No	551 (57)	360 (77)	100 (30)	71 (75)	20 (30)	
Yes	413 (43)	106 (23)	237 (70)	24 (25)	46 (70)	
Tumor location — no./total no. (%)						0.13
Rectum	212/961 (22)	118/465 (25)	70/335 (21)	14/95 (15)	10/66 (15)	
Distal colon	313/961 (33)	148/465 (32)	114/335 (34)	29/95 (31)	22/66 (33)	
Proximal colon	436/961 (45)	199/465 (43)	151/335 (45)	52/95 (55)	34/66 (52)	
Disease stage — no. (%)						0.01
I	260 (27)	112 (24)	102 (30)	19 (20)	27 (41)	
II	301 (31)	159 (34)	87 (26)	36 (38)	19 (29)	
III	264 (27)	128 (27)	99 (29)	23 (24)	14 (21)	
IV	64 (7)	31 (7)	18 (5)	12 (13)	3 (5)	
Unknown	75 (8)	36 (8)	31 (9)	5 (5)	3 (5)	

Table 1. (Continued.)

Characteristic	All Patients (N=964)	Wild-Type *PIK3CA*		Mutant *PIK3CA*		P Value
		No Aspirin Use (N=466)	Aspirin Use (N=337)	No Aspirin Use (N=95)	Aspirin Use (N=66)	
Tumor differentiation — no./total no. (%)						0.85
Well or moderately differentiated	880/958 (92)	422/461 (92)	311/337 (92)	85/94 (90)	62/66 (94)	
Poorly differentiated	78/958 (8)	39/461 (8)	26/337 (8)	9/94 (10)	4/66 (6)	
Microsatellite instability — no./total no. (%)						0.84
None or low level	800/952 (84)	382/460 (83)	283/332 (85)	81/95 (85)	54/65 (83)	
High level	152/952 (16)	78/460 (17)	49/332 (15)	14/95 (15)	11/65 (17)	
CIMP — no./total no. (%)						0.84
Low or negative	755/916 (82)	366/438 (84)	264/323 (82)	75/93 (81)	50/62 (81)	
High	161/916 (18)	72/438 (16)	59/323 (18)	18/93 (19)	12/62 (19)	
BRAF — no./total no. (%)						0.94
Wild-type	828/959 (86)	400/464 (86)	288/335 (86)	84/95 (88)	56/65 (86)	
Mutant	131/959 (14)	64/464 (14)	47/335 (14)	11/95 (12)	9/65 (14)	
KRAS — no./total no. (%)						<0.001
Wild-type	623/959 (65)	317/463 (68)	231/336 (69)	44/95 (46)	31/65 (48)	
Mutant	336/959 (35)	146/463 (32)	105/336 (31)	51/95 (54)	34/65 (52)	
LINE-1 methylation level — %	62.9±9.5	62.7±10.0	62.4±9.2	64.2±9.4	64.3±9.4	0.58
PTGS2 expression — no./total no. (%)						0.12
Negative	315/798 (39)	149/395 (38)	104/276 (38)	37/79 (47)	25/48 (52)	
Positive	483/798 (61)	246/395 (62)	172/276 (62)	42/79 (53)	23/48 (48)	
Phosphorylated AKT — no./total no. (%)						0.02
Negative	195/562 (35)	102/266 (38)	73/200 (36)	11/58 (19)	9/38 (24)	
Positive	367/562 (65)	164/266 (62)	127/200 (63)	47/58 (81)	29/38 (76)	

* Plus–minus values are means ±SD. Aspirin use was defined as regular use of aspirin during most weeks, whereas aspirin nonuse was defined as no regular use of aspirin during most weeks. CIMP denotes CpG island methylator phenotype, and LINE-1 long interspersed nucleotide element 1.

† Data for men were obtained from the Health Professionals Follow-up Study, and data for women were obtained from the Nurses' Health Study.

‡ The body-mass index is the weight in kilograms divided by the square of the height in meters.

STATISTICAL ANALYSIS

A detailed description of the statistical analysis is provided in the Supplementary Appendix. All reported P values are two-sided. There was no prespecified subgroup analysis. We performed 13 post hoc subgroup analyses (for two different end points); the results of 12 of these analyses are reported here (Table S1 in the Supplementary Appendix). Thus, up to two false positive findings would be expected by chance alone. The Kaplan–Meier method and log-rank test were performed for the survival analysis. In the analyses of colorectal cancer–specific mortality, data on deaths from causes other than colorectal cancer were censored. To control for confounding, we used Cox proportional-hazards models to calculate the hazard ratio for death according to aspirin use or nonuse and the presence or absence of tumor *PIK3CA* mutation. The significance of an interaction was assessed by means of the Wald test on the cross-product term of the aspirin and *PIK3CA* variables.

RESULTS

PATIENTS

Table 1 summarizes the baseline characteristics of the 964 patients with colorectal cancer, according to aspirin use or nonuse after diagnosis and the presence or absence of tumor *PIK3CA* mutation. *KRAS* mutations were associated with mu-

tated-*PIK3CA* tumors, as previously described.[15] As compared with patients who did not use aspirin before diagnosis, a higher proportion of patients who used aspirin before diagnosis also used it after diagnosis, as previously described.[8] The proportion of mutated-*PIK3CA* tumors was 17% (in 70 of 413 patients) among patients who used aspirin before diagnosis and 17% (in 91 of 551 patients) among patients who did not use aspirin before diagnosis. There were 395 deaths overall, including 190 deaths from colorectal cancer. The median follow-up was 153 months (interquartile range, 104 to 195) among patients with data that were censored.

ASPIRIN USE AND SURVIVAL ACCORDING TO *PIK3CA* MUTATION STATUS

We tested the hypothesis that the effect of postdiagnosis use of aspirin on survival might be stronger in mutated-*PIK3CA* colorectal cancer than in wild-type *PIK3CA* cancer (Fig. 1 and Table 2, and Table S2 in the Supplementary Appendix). Among patients with mutated-*PIK3CA* tumors, regular use of aspirin after diagnosis was associated with significantly longer cancer-specific survival (multivariate hazard ratio for cancer-related death, 0.18; 95% confidence interval [CI], 0.06 to 0.61; P<0.001 by the log-rank test). In contrast, among patients with wild-type *PIK3CA* tumors, regular use of aspirin after diagnosis was not associated with cancer-specific survival (multivariate hazard ratio for death, 0.96; 95% CI, 0.69 to 1.32; P=0.76 by the log-rank test; P=0.009 for the interaction). Although statistical power was limited, the effect of aspirin on survival among patients with mutated-*PIK3CA* tumors appeared consistent irrespective of the dose (data not shown).

Because only three colorectal cancer–specific deaths occurred among 66 patients with mutated-*PIK3CA* tumors who used aspirin after diagnosis, the log-rank or Cox regression analysis may not have yielded a robust P value. We therefore used Fisher's exact test to examine the association between aspirin use after diagnosis and 5-year survival according to the presence or absence of tumor *PIK3CA* mutation (Table 3). Among patients with mutated-*PIK3CA* tumors, 23 of 90 patients who did not use aspirin after diagnosis (26%) died within 5 years after diagnosis, whereas only 2 of 62 regular users of aspirin after diagnosis (3%) died within 5 years after diagnosis (P<0.001). In contrast, among patients with wild-type *PIK3CA* tumors, the 5-year cumulative colorectal cancer–specific mortality was the same (15%) for users and nonusers of aspirin after diagnosis (P=0.92).

We obtained information on aspirin use after diagnosis with the use of a biennial questionnaire, and some patients with stage IV disease did not survive long enough to be included in our analysis; this might have resulted in selection bias in favor of patients with indolent stage IV disease. Therefore, we performed a sensitivity analysis limited to patients with stage I, II, or III disease (excluding patients with stage IV disease), which yielded similar results (Table S3 in the Supplementary Appendix). Results of analyses limited to stage II or III disease and to stage III disease alone are included in Tables S4 and S5 in the Supplementary Appendix, respectively.

We performed an exploratory analysis to determine whether aspirin use before diagnosis might have modified the interactive prognostic association between aspirin use after diagnosis and tumor *PIK3CA* mutation (Table 4, and Table S6 in the Supplementary Appendix). Among patients with wild-type *PIK3CA* tumors, regular use of aspirin after diagnosis was not significantly associated with colorectal cancer–specific or overall survival, irrespective of aspirin use before diagnosis. The small number of deaths among patients with mutated-*PIK3CA* tumors who used aspirin after diagnosis precluded robust statistical assessments.

ASPIRIN USE AND SURVIVAL ACCORDING TO *PIK3CA* MUTATION AND PTGS2 EXPRESSION STATUS

Our previous study[8] showed that regular use of aspirin after diagnosis was associated with reduced mortality from colorectal cancer, especially among patients with PTGS2-positive tumors. Thus, as a further exploratory analysis, we examined the relationship between aspirin use after diagnosis and survival according to combined PTGS2 and *PIK3CA* status (Table S7 in the Supplementary Appendix). In patients with wild-type *PIK3CA* tumors, aspirin use after diagnosis, even among those with PTGS2-positive tumors, did not appear to be significantly associated with survival. However, the effect of aspirin appeared to be stronger among patients with both mutated-*PIK3CA* and PTGS2-positive tumors, although the number of events in patients with mutated-*PIK3CA* tumors was too small to perform a robust statistical analysis.

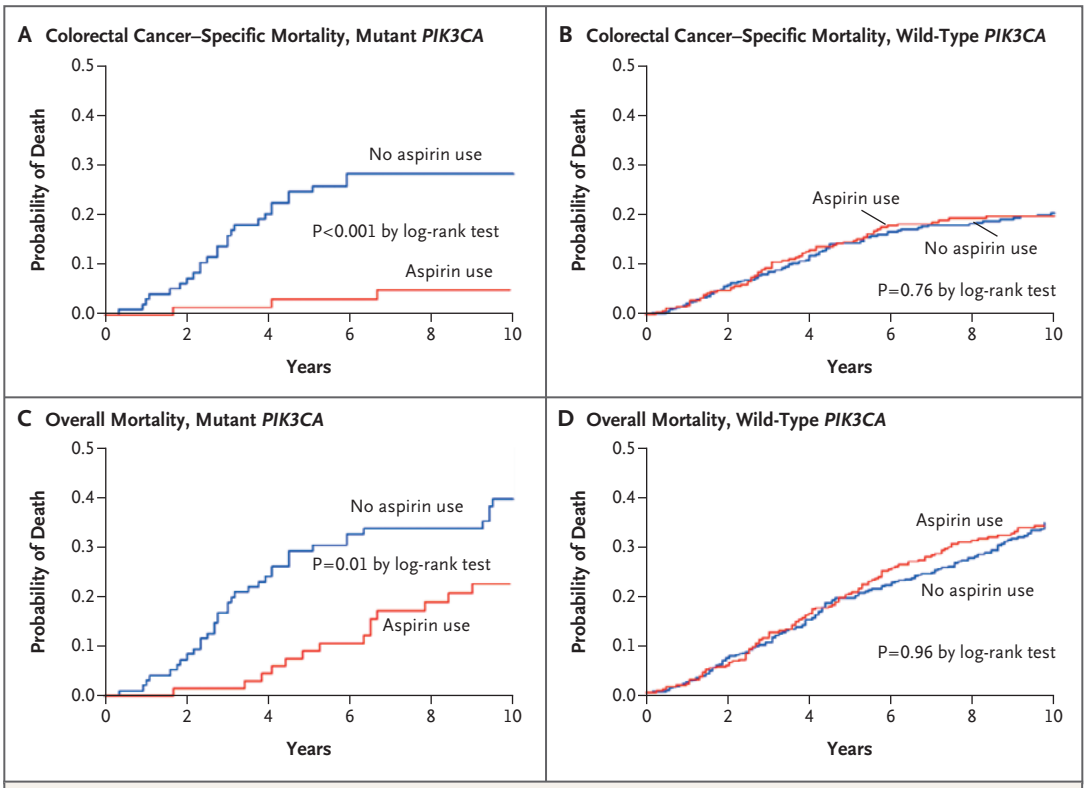

Figure 1. Mortality among Patients with Colorectal Cancer, According to Regular Use or Nonuse of Aspirin after Diagnosis and *PIK3CA* Mutation Status.

Panels A and B show colorectal cancer–specific mortality among patients with mutant-*PIK3CA* tumors and those with wild-type *PIK3CA* tumors, respectively, and Panels C and D show overall mortality in the respective subgroups of patients.

ASPIRIN USE AND SURVIVAL ACCORDING TO *PIK3CA* MUTATION STATUS AND OTHER SELECTED VARIABLES

We conducted exploratory analyses of aspirin use after diagnosis and patient survival according to *PIK3CA* mutation status and other selected variables, limiting these analyses to patients with microsatellite-stable cancer. The results (Table S8 in the Supplementary Appendix) are similar to the results of the primary analysis (Table 2).

Next, we performed an analysis stratified according to the status of phospho-AKT expression (Table S9 in the Supplementary Appendix). Also, because the frequency of *PIK3CA* mutation may gradually increase along colorectal subsites from the rectum to the ascending colon,[33] we performed an analysis according to tumor location (Table S10 in the Supplementary Appendix). However, in both analyses, statistical power was limited because of the small number of deaths among patients with mutated-*PIK3CA* tumors.

NSAID USE AND SURVIVAL ACCORDING TO *PIK3CA* MUTATION STATUS

We considered the possibility that concurrent use of a nonsteroidal antiinflammatory drug (NSAID) may have confounded our associations. However, inclusion of regular use of NSAIDs in our multivariate models did not materially alter our effect estimates for the association between aspirin use after diagnosis and survival. In exploratory analyses of regular use of NSAIDs after diagnosis and survival according to the presence or absence of *PIK3CA* mutation (Table S11 in the Supplementary Appendix), there was no significant interaction between NSAID use and *PIK3CA* status (P=0.48 for interaction).

DISCUSSION

We found that tumor *PIK3CA* mutation and regular use of aspirin after diagnosis had a significant

Table 2. Hazard Ratios for Death with Adjustment for Stage of Disease and Other Variables, According to Tumor *PIK3CA* Mutation Status and Use or Nonuse of Aspirin after Diagnosis.*

PIK3CA	Total No. of Patients	Colorectal Cancer–Specific Mortality				
		No. of Deaths	Univariate Hazard Ratio (95% CI)	P Value†	Hazard Ratio, Adjusted for Disease Stage (95% CI)	P Value†
Wild-type				0.003		0.01
No aspirin use	466	96	1.00 (referent)		1.00 (referent)	
Aspirin use	337	65	0.95 (0.69–1.30)		0.93 (0.68–1.28)	
Mutant						
No aspirin use	95	26	1.00 (referent)		1.00 (referent)	
Aspirin use	66	3	0.14 (0.04–0.47)		0.18 (0.05–0.60)	

* The multivariate Cox regression model, stratified according to and adjusted for disease stage, initially included age, sex, year of diagnosis, time from diagnosis to first measurement of aspirin use after diagnosis (in months), regular use or nonuse of aspirin before diagnosis, tumor location, tumor differentiation, body-mass index, microsatellite instability status, CpG island methylator phenotype, *KRAS* mutation, *BRAF* mutation, LINE-1 (long interspersed nucleotide element-1) methylation, and the presence or absence of PTGS2 expression. A backward-elimination model with a threshold of P=0.05 was used to select variables in the final models. CI denotes confidence interval.

† P values are for the interaction of aspirin use and *PIK3CA* mutation.

interactive effect on survival among patients with colorectal cancer. Specifically, among patients with mutated-*PIK3CA* tumors, regular use of aspirin after diagnosis was associated with significantly increased survival. In contrast, patients with wild-type *PIK3CA* tumors did not appear to derive a benefit from aspirin use after diagnosis. In addition, the effect of aspirin appeared to be most pronounced in patients who had tumors with both *PIK3CA* mutation and PTGS2 expression. Our data support the hypothesis that aspirin use after diagnosis may have a differential effect on survival, depending on the presence or absence of tumor *PIK3CA* mutation.

Our data suggest that regular use of aspirin is suitable for testing as an adjuvant treatment in patients with mutated-*PIK3CA* cancers and that *PIK3CA* mutation status may serve as a tumor biomarker that predicts the response to adjuvant aspirin treatment. Our data also suggest that even relatively low doses of aspirin may prolong survival among patients with mutated-*PIK3CA* cancer. Nevertheless, because of the small numbers of deaths in some subgroups, we must be cautious in interpreting our data. Furthermore, since our current analysis was not prespecified when the cohorts were initially enrolled, and testing of multiple hypotheses through subgroup analyses increases the possibility of a false positive result,[34] our findings need to be confirmed by analyses of independent data sets.

A possible alternative explanation for our findings is that aspirin use before diagnosis, which is related to aspirin use after diagnosis, may be associated with the occurrence of indolent tumor subtypes, particularly among mutated-*PIK3CA* tumors. We previously reported that aspirin use before diagnosis by itself was not associated with prognosis among patients with colorectal cancer.[8] In our current analysis, we analyzed the effect of aspirin use after diagnosis according to both *PIK3CA* mutation and aspirin use before diagnosis (Table 3). Although statistical power was limited, the results suggest that the association between aspirin use after diagnosis and increased survival is probably not explained by aspirin use before diagnosis. Colorectal cancers are a heterogenous group of complex diseases, as indicated by molecular pathological epidemiology[35-37] and the unique tumor principle.[11] Thus, it is not possible to explain tumor behavior on the basis of one or a few biomarkers alone. The interplay among inflammation, aspirin, and tumor molecular features is suggested by the current study and our previous studies.[8,22]

The proportion of mutated-*PIK3CA* tumors was the same (17%) among users and nonusers of aspirin before diagnosis, despite our main finding that aspirin use after diagnosis appeared to prevent progression of disease in patients with mutated-*PIK3CA* tumors. One reason for this apparent discrepancy may be related to tumor evolution.

		Overall Mortality					
Multivariate Hazard Ratio, Adjusted for Disease Stage (95% CI)	P Value†	No. of Deaths	Univariate Hazard Ratio (95% CI)	P Value†	Hazard Ratio, Adjusted for Disease Stage (95% CI)	P Value†	Multivariate Hazard Ratio, Adjusted for Disease Stage (95% CI)	P Value†
	0.009			0.02		0.06		0.07
1.00 (referent)		196	1.00 (referent)		1.00 (referent)		1.00 (referent)	
0.96 (0.69–1.32)		137	1.01 (0.81–1.25)		1.01 (0.81–1.25)		0.94 (0.75–1.17)	
1.00 (referent)		44	1.00 (referent)		1.00 (referent)		1.00 (referent)	
0.18 (0.06–0.61)		18	0.49 (0.28–0.85)		0.57 (0.33–0.98)		0.54 (0.31–0.94)	

During tumor evolution, tumor cells are subject to changes in their own genome, epigenome, proteome, and metabolome and to changes in the local microenvironment.[11] Thus, their dependence on an inflammatory microenvironment probably varies according to the specific phase of tumor evolution, which may result in the differential interaction of aspirin use and *PIK3CA* mutation in the early phase of evolution (before diagnosis) versus the late phase (after diagnosis).

Our previous data[8] suggested that patients who use aspirin before diagnosis may not benefit from aspirin use after diagnosis. However, our current study provides evidence of a beneficial effect of aspirin use after diagnosis if the colorectal cancer has *PIK3CA* mutation, irrespective of aspirin use or nonuse before diagnosis. This finding may prove to have substantial implications for decisions about treatment.

In our current study, the strongest effect of aspirin use was in patients who had tumors with both *PIK3CA* mutation and PTGS2 expression. We must interpret these results with caution, however, because of the multiple subgroup analyses and limited statistical power. Nonetheless, our current findings are not inconsistent with those of our previous study,[8] which showed a strong antitumor effect of aspirin on PTGS2-positive colorectal cancer. Experimental evidence supports cross-talk between the PI3K and PTGS2 pathways.[20,21] In combination with the experimental observation that aspirin can induce cell apoptosis through PTGS2-independent pathways,[38,39] our data may provide support for an antitumor effect of aspirin in addition to that of PTGS2 inhibition, although the exact mechanisms need to be clarified.

A "colorectal continuum" hypothesis that is distinctive from the long-standing "proximal versus distal colorectum" model has recently been proposed.[33,40] The frequencies of molecular features such as a high level of microsatellite instability and extensive CpG island methylation, as well as *BRAF* and *PIK3CA* mutations, appear to increase continuously from the rectum to ascending colon.[33] Considering a gradual transition of gut biogeography, the inhibitory effect of aspirin on cancer may differ according to both the specific site of the tumor and its molecular features. Our current study lacked statistical power to examine effect modification according to both *PIK3CA* mutation status and the specific tumor site, and larger studies should address this question.

Our study has several strengths. We collected data on aspirin use both before diagnosis and after diagnosis; this allowed us to examine the potential influence of the timing of aspirin use in re-

Table 3. Colorectal Cancer–Specific Survival at 5 Years, According to Tumor *PIK3CA* Mutation Status and Use or Nonuse of Aspirin after Diagnosis.*

PIK3CA	No. of Patients	Dead at 5 Yr after Diagnosis	Alive at 5 Yr after Diagnosis	P Value
		number (percent)		
Wild-type				0.92
No aspirin use	440	65 (15)	375 (85)	
Aspirin use	319	48 (15)	271 (85)	
Mutant				<0.001
No aspirin use	90	23 (26)	67 (74)	
Aspirin use	62	2 (3)	60 (97)	

* P values were calculated with the use of Fisher's exact test.

Table 4. Colorectal-Cancer Mortality, According to Tumor *PIK3CA* Mutation Status and Aspirin Use or Nonuse before Diagnosis and after Diagnosis.*

Variable	No. of Patients	Colorectal Cancer–Specific Mortality			Overall Mortality			
		No. of Events	Univariate Hazard Ratio (95% CI)	Hazard Ratio, Adjusted for Disease Stage (95% CI)	Multivariate Hazard Ratio, Adjusted for Disease Stage (95% CI)	No. of Events	Univariate Hazard Ratio (95% CI)	Hazard Ratio, Adjusted for Disease Stage (95% CI)	Multivariate Hazard Ratio, Adjusted for Disease Stage (95% CI)
Wild-type *PIK3CA*									
No aspirin use before diagnosis									
No aspirin use after diagnosis	360	71	1 (referent)	1 (referent)	1 (referent)	147	1 (referent)	1 (referent)	1 (referent)
Aspirin use after diagnosis	100	17	0.84 (0.49–1.43)	0.82 (0.48–1.39)	0.90 (0.53–1.54)	41	0.99 (0.70–1.39)	0.98 (0.69–1.39)	0.97 (0.68–1.37)
Aspirin use before diagnosis									
No aspirin use after diagnosis	106	25	1 (referent)	1 (referent)	1 (referent)	49	1 (referent)	1 (referent)	1 (referent)
Aspirin use after diagnosis	237	48	0.88 (0.54–1.43)	0.93 (0.57–1.52)	0.92 (0.56–1.51)	96	0.92 (0.66–1.31)	0.96 (0.68–1.36)	0.85 (0.60–1.21)
Mutant *PIK3CA*									
No aspirin use before diagnosis									
No aspirin use after diagnosis	71	20	1 (referent)	1 (referent)	1 (referent)	34	1 (referent)	1 (referent)	1 (referent)
Aspirin use after diagnosis	20	1	0.16 (0.02–1.17)	0.20 (0.03–1.46)	0.28 (0.04–2.10)	6	0.57 (0.24–1.35)	0.63 (0.26–1.51)	0.59 (0.24–1.41)
Aspirin use before diagnosis									
No aspirin use after diagnosis	24	6	1 (referent)	1 (referent)	1 (referent)	10	1 (referent)	1 (referent)	1 (referent)
Aspirin use after diagnosis	46	2	0.15 (0.03–0.74)	0.22 (0.04–1.10)	0.18 (0.04–0.92)	12	0.54 (0.23–1.24)	0.68 (0.29–1.58)	0.60 (0.26–1.40)

* The multivariate Cox regression model, stratified according to and adjusted for disease stage, initially included age, sex, year of diagnosis, time from diagnosis to first measurement of aspirin use after diagnosis (in months), regular use or nonuse of aspirin before diagnosis, tumor location, tumor differentiation, body-mass index, microsatellite instability status, CpG island methylator phenotype, *KRAS* mutation, *BRAF* mutation, LINE-1 (long interspersed nucleotide element-1) methylation, and the presence or absence of PTGS2 expression. A backward-elimination model with a threshold of P=0.05 was used to select variables in the final models.

lation to the cancer diagnosis. Since all study participants were health professionals with knowledge of medications, the accuracy of self-reported information on aspirin use was probably high. Furthermore, our comprehensive molecular pathological epidemiology database,[35-37] with accumulated data on various lifestyle factors, tumor molecular features, and clinical outcomes, provided a unique opportunity to test our specific hypothesis of the interactive prognostic effect between aspirin use and *PIK3CA* mutation.

Our current study also has some limitations. Data on cancer treatment were limited. Given that all patients received a diagnosis before July 1, 2006, we assume that chemotherapy use did not differ substantially according to *PIK3CA* mutation status, information that was unavailable to the treating physicians. Moreover, our multivariable survival analysis was adjusted for cancer stage (I, II, III, or IV), and decision making for treatment was largely based on the stage of the cancer. In addition, we had limited information about cancer recurrence; nevertheless, with adequate follow-up time in the current study, colorectal cancer–specific survival served as a reasonable measure of the colorectal cancer–specific outcome.

In conclusion, this study suggests that regular use of aspirin after the diagnosis of colorectal cancer is significantly associated with increased survival among patients with mutated-*PIK3CA* tumors but not among patients with wild-type *PIK3CA* tumors. This relationship appeared to be independent of aspirin use before diagnosis. *PIK3CA* mutation may serve as a tumor biomarker that predicts the response to the initiation of aspirin therapy in patients with newly diagnosed colorectal cancer.

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