Aspirin and colorectal cancer: Back to the Future

David Tougeron[1], **Dan Sha**[3], **Sashidhar Manthravadi**[1], and **Frank A. Sinicrope**[1,2,3]

[1]Department of Medicine, Rochester, MN

[2]Department of Oncology, Rochester, MN

[3]Mayo Clinic and Mayo Cancer Center, Rochester, MN

Abstract

Abundant epidemiological evidence indicates that regular and long term use of aspirin is associated with a significant reduction in the incidence of colorectal cancer (CRC). The long duration of aspirin needed to prevent CRC is believed to be due to inhibition of precursor lesions known as adenomas, whose recurrence is inhibited by aspirin in randomized trials. Aspirin intake has also been associated with a statistically significant improvement in patient survival after curative resection of CRC in large observational studies. In these cohorts, the survival benefit of aspirin was shown to depend upon the level of cyclooxygenase-2 (COX-2) expression in the primary CRC. More recent analysis of patient tumors from these observational cohorts suggests that the benefit of aspirin may be limited to specific molecular subtypes. Aspirin intake following CRC resection was associated with a significant improvement of survival in patients whose tumors carried mutant, but not wild-type, copies of the *phosphatidylinositol 3-kinase* (*PI3KCA*) gene, especially tumors that overexpressed COX-2. A mechanistic explanation is suggested by the finding that inhibition of COX-mediated prostaglandin E2 synthesis by aspirin attenuates *PI3K* signaling activity that is known to regulate cancer cell proliferation and survival. Aspirin has also been shown to reduce the incidence of CRCs bearing wild-type, but not mutant alleles of the *BRAF*^{V600E} oncogene. While provocative, the potential utility of these molecular markers for predicting aspirin efficacy awaits prospective evaluation in clinical trials. If validated, these finding may support a personalized approach to using aspirin for the therapy of CRC.

Keywords

Aspirin; COX-2; colorectal cancer; BRAF; PIK3Ca

Acetylsalicylic acid was first synthesized in 1853 and used for its analgesic and anti-inflammatory properties. Aspirin acts on cyclooxygenase (COX) enzymes that regulate the synthesis of prostaglandins (PGs) and related eicosanoids from arachidonic acid (Fig. 1). It inhibits constitutively expressed COX-1 as well as the COX-2 isoform which is upregulated at sites of inflammation (1). Selective COX-2 inhibitors were developed to reduce gastrointestinal injury but were later found to have cardiovascular toxicities (2). Large observational studies have demonstrated an association of regular and long-term aspirin intake with a significant reduction in the incidence (3–5) and mortality from colorectal cancer (CRC) (3, 6). Cohorts from the Nurses' Health Study (NHS) and the Health Professionals Follow-up Study (HPFS) of 130,274 total participants provided data on aspirin use from a questionnaire administered every 2 years. Among these cohorts, there were 636

Corresponding Author: Frank A. Sinicrope, M.D., Mayo Clinic, 200 First St. SW, Rochester, MN 55905. Sinicrope.frank@mayo.edu Tel: 507-255-5713. Fax: 507 255 6318.

incident CRCs of which 67% were found to overexpress COX-2 proteins when analyzed retrospectively. Regular use of aspirin (two 325-mg tablets per week) was shown to significantly reduce the incidence of CRCs overexpressing COX-2 (relative risk (RR)=0.64; 95%CI, 0.52–0.78; P=0.02), but not those with weak or absent COX-2 expression in the primary tumor (Table 1). Importantly, the ability of aspirin to reduce CRC incidence became evident only after regular use for more than 10 years (multivariate RR=0.59; 95%CI, 0.42 to 0.82; P for trend <0.001). The relative risk was further reduced as the number of aspirin tablets (325 mg) taken per week increased (0.5–1.5 *vs* 2–5 *vs* 6–14 *vs* >14; P_{trend}=0.001), indicating that the chemopreventive effect was dependent upon both the dose and duration of aspirin intake (7), suggesting the importance of cumulative dosage as a determinant of aspirin efficacy in these settings.

The explanation as to why a prolonged duration of aspirin intake, varying between studies from 4 years to greater than 10 years (5, 8), is needed to reduce the incidence of CRC is likely due to a chemopreventive effect of aspirin on colorectal adenomas that are precursor lesions of CRC. In preclinical models, aspirin inhibits the development of colorectal adenomas and their progression to carcinoma (9). Using colorectal adenomas as a surrogate end point for CRC, earlier randomized and controlled trials of aspirin for the chemoprevention of CRC were negative(10, 11); however, more recent randomized trials have consistently demonstrated aspirin's ability to decrease adenoma recurrence in patients with prior colorectal adenomas or cancer (12, 13), although the minimally effective dose remains unclear (14). The failure of earlier studies to detect a chemopreventive effect of aspirin may be due, in part, to the need for prolonged follow-up as studies reporting no reduction in CRC incidence initially (11, 15, 16) often noticed an effect after a longer interval ranging from 56 months to around 17 years (8, 17). A recent study involving 39,876 women aged 45 years or older who were enrolled in the Women's Health Study found that alternate day dosing of low dose aspirin (100 mg) taken for 10 or more years significantly reduced the incidence of CRC in women (HR=0.80; 95% CI, 0.67–0.97; P=0.021), especially in the proximal colon (17). After 18 years, the incidence of CRC was 20% lower in the aspirin group than in the placebo group and was accompanied by a significant increase in self-reported gastrointestinal toxicities (HR for GI bleeding 1.14; 95% CI, 1.06–1.22; P <0.001). In a high-risk population, i.e., patients with prior colon cancer, a prospective study involving 635 participants found that treatment with 325 mg/day aspirin over a mean duration of 30.9 months was associated with a statistically significant reduction in the risk of recurrent colorectal adenomas (13). Similar to aspirin, the selective COX-2 inhibitor, celecoxib, has been shown to effectively reduce adenoma recurrence in patients with prior adenomas in randomized trials (18, 19). In a 20 year follow-up of 5 randomized trials, aspirin at doses of at least 75 mg daily taken for several years reduced the long-term incidence and mortality from CRC, with the benefit being greatest for cancers of the proximal colon (3). The tumor-site related efficacy of aspirin is clinically important in that colonoscopy has been shown to be less effective at preventing right-sided *vs* left-sided colon cancers (20). Data also exist for the chemopreventive efficacy of aspirin in patients with Lynch Syndrome (LS) who have an 80% lifetime risk of CRCs that develop via defective DNA mismatch repair (MMR) (21). Long-term aspirin treatment (600 mg/day for >2 years) was shown to significantly reduce the incidence of CRCs (N=508) (HR= 0.41; 95% CI, 0.19–0.86; P=0.02) in LS patients during prolonged follow-up (mean 55·7 months; range 1–128)(8).

In a recent report of patients within the NHS and HPFS who developed CRC, a potential predictive biomarker for aspirin efficacy was found. Regular aspirin intake was associated with a significant reduction in the incidence of CRCs with wild-type (WT), but not mutant $BRAF^{V600E}$ (Table 1)(22). Detailed analysis of the patients who tumors carried WT-*BRAF* revealed that the preventive benefit of aspirin appeared to be concentrated in tumors

overexpressing COX-2 proteins (multivariable HR 0.67; 95% CI 0.56–0.81; P=0.018). In contrast, aspirin failed to lower the risk of $BRAF^{V600E}$-mutated CRCs irrespective of their level of COX-2 expression. Protection conferred by aspirin against the development of $BRAF$-WT CRCs was not abrogated by mutations in $PIK3CA$ exons 9 and 20 or $KRAS$ exon 2 (22). Furthermore, aspirin benefit was unrelated to MSI status in incident CRCs in the NHS and HPFS cohorts (A.T. Chan, *personal communication*). Of note, chemopreventive efficacy for aspirin was seen in CRCs from LS patients that almost uniformly carry WT copies of $BRAF$ (23), whereas activating mutations in the $BRAF^{V600E}$ oncogene, detected in up to 15% of CRCs, are enriched in sporadic CRCs with defective MMR and microsatellite instability (MSI) due to epigenetic inactivation of $MLH1$ MMR gene (24, 25). COX-2 inhibition was unable to suppress proliferation in $KRAS$-mutated cells which suggests that this may also be the case in $BRAF^{V600E}$ mutated CRC cells (26). The finding that aspirin can selectively reduce the incidence of $BRAF$-WT CRCs awaits prospective validation, and studies to identify the mechanism underlying its potential predictive impact are awaited.

In addition to its role in the prevention of CRC, data also indicate a role for aspirin as an adjuvant agent in patients with resected CRC (Table 2). Compelling data were obtained from the NHS and HPFS studies (27) where 1279 patients with non-metastatic CRCs were identified retrospectively, and then categorized based on aspirin usage post diagnosis (two 325-mg tablets per week). During a median follow-up of 11.8 years from diagnosis, aspirin use post diagnosis (N=549) was associated with a statistically significant reduction in both CRC–specific mortality(HR=0.71; 95% CI 0.53–0.95, P=0.02) and overall mortality (HR=0.79; 95% CI 0.65–0.97, P=0.03) compared to non-aspirin users. Stratifying tumors based on expression of COX-2 revealed that the survival benefit from aspirin use was confined to patients whose primary tumors overexpressed COX-2 proteins (CRC-specific multivariate HR=0.39; 95%CI 0.20–0.76). In contrast, a subgroup analysis of study participants who reported aspirin use prior to a CRC diagnosis indicated no mortality reduction even when aspirin use was continued post-diagnosis (n=21)($P_{interaction}$= 0.09)(27), suggesting that exposure to aspirin pre-diagnosis may select for aspirin-resistant tumor cells. In an earlier study, the same investigators reported a post-hoc analysis of a subgroup of stage III colon cancer patients enrolled in an adjuvant chemotherapy trial (CALGB 89803) where aspirin users had lower rates of colon cancer recurrence and death compared to non-users(28). Among 2916 patients with CRC identified from the Health Informatics Centre registry in Scotland, aspirin use(median of 1.53 years) post-diagnosis was associated with improved CRC-specific survival (multivariate HR=0.58; 95% CI 0.45–0.75, P<0.001) (29) (Table 2). Similarly, a cancer registry study conducted in the Netherlands identified 1451 patients with CRC in whom aspirin use post-diagnosis(defined as physician-prescribed aspirin for at least 14 days) conferred a statistically significant survival benefit in patients with colon cancer (HR=0.62; 95%CI 0.48–0.80; P<0.001), but not rectal cancer (30). Further support for an effect of aspirin on micrometastases derives from 5 different randomized trials of aspirin for the prevention of vascular events that showed that aspirin use (80–325 mg/day) decreased the risk of metastases from CRC at diagnosis, as well as the risk of subsequent metastasis at follow-up in patients who were initially metastasis-free (HR=0.26; 95% CI 0.11–0.57; P=0.0008)(31). As was seen for its chemopreventive effects, the presumed anti-metastatic effects of aspirin also appear to be dose-dependent in that an increase in post-diagnosis aspirin dosage from 0.5–5 to >6 tablets per week led to a modest improvement in survival benefit In the NHS and HPFS patient cohorts (P_{trend}=0.04) (27). Taken together, these studies suggest that aspirin warrants further evaluation as an adjuvant agent to eradicate micrometastases. In this regard, the ASCOLT study is the first prospective randomized placebo-controlled trial to evaluate aspirin as adjuvant therapy in resected CRC. In this study, 200 mg aspirin is administered daily for 3 years as adjuvant treatment in patients with resected stage III or high risk stage II CRC (32).

Recent data suggest the potential utility of *PIK3CA* mutation status in CRCs for the prediction of clinical benefit from aspirin in the adjuvant setting. Mutations in the *PIK3CA* gene are detected in 15–20% of CRCs (33) and lead to constitutive activation of the PI3K-Akt pathway. An uncertain role exists for *PIK3CA* mutations in prognosis (34) and in predicting resistance to anti-EGFR targeted therapy (35, 36). A retrospective analysis of CRC patients from the NHS and HPFS cohorts detected *PIK3CA* mutations in 161 of 964 (17%) non-metastatic tumors. Patients were then categorized based on aspirin usage post-diagnosis and at a median follow-up of 153 months, aspirin intake (two 325-mg tablets per week) in *PIK3CA* mutation carriers was associated with a statistically significant increase in survival (multivariate HR=0.18; 95% CI 0.06–0.61; p<0.001), whereas patients whose tumors had WT alleles (N=803) did not derive any benefit (Table 1) (37). Among patient tumors with *PIK3CA* mutations, the survival benefit associated with aspirin was most evident in tumors with overexpression of COX-2 [N=55/161 (34%)]. Similar findings were recently reported in a post-hoc analysis of a clinical trial (VICTOR) evaluating rofecoxib as adjuvant therapy of stage II and III colon cancers (38). Patient tumors were categorized by *PIK3CA* mutation status and aspirin usage was recorded at the time of study enrollment. Patients taking < 100 mg of aspirin daily were not excluded and were allowed to continue this therapy during the clinical trial. In patients whose tumors carried *PIK3CA* mutations (N=104), aspirin usage (n=14) was associated with a statistically significant improvement in recurrence-free survival (RFS) (multivariate HR=0.11, 95% CI, 0.001–0.832; P=0.027) at a median follow-up of 61.5 months (38). A modest improvement in overall survival (OS) was also found that did not reach statistical significance (multivariate HR=0.29, 95% CI, 0.04–2.330; P= 0.260). In contrast, rofecoxib treatment was not associated with a difference in RFS (multivariate HR = 1.22; 95% CI, 0.50–2.98; P= 0.473). While the duration of aspirin use prior to study enrollment was not reported, the median duration of rofecoxib use was 7.4 months. Despite the pronounced survival benefit of aspirin in *PIK3Ca* mutation carriers observed in the VICTOR trial, these data derive from a very small number of patients who reported aspirin usage. The lack of efficacy of the selective COX-2 inhibitor rofecoxib, suggests that inhibition of constitutive COX-1 may be mechanistically important. Furthermore, the ability of aspirin to inhibit platelet aggregation that is mediated by COX-1, may be important in its anti-tumor effect (39).

Acting through its cell surface receptors EP1–EP4, PGE_2 regulates cellular processes important in cancer development (Fig. 1). PGE_2 acts through EP4 to activate Tcf/Lef signaling through a PI3-kinase-dependent pathway (40). Inhibition of PGE_2 signaling by aspirin may therefore, attenuate PI3K activity in *PIK3CA* mutant cancers (41). In addition to inhibiting PGE_2, aspirin has been shown to inhibit mTOR, a downstream effector of the PI3K pathway by activation of AMPK (Adenosine Monophosphate-activated Protein Kinase) in CRC cells (42). The mechanisms underlying the anti-tumor properties of aspirin include both COX-dependent and -independent effects (43). PGE_2 stimulates angiogenesis by induction of VEGF (Vascular endothelial growth factor) and bFGF (basic Fibroblast growth factor)(44), and can modulate the WNT/β-catenin pathway to enable an epithelial-to-mesenchymal transition, a critical event for metastasis (45). COX-independent mechanisms contribute to the anti-tumor effects of aspirin by inhibiting PPARδ (peroxisome proliferator-activated receptor) (46) and the NF-κB pathway (47–49). Aspirin can also exert immunomodulatory effects by altering chemokines (CCL2 and CXCL10) that lead to decreased numbers of myeloid-derived suppressor cells (MDSCs) and an increase in cytotoxic CD8+ T-cells (50). Both aspirin and selective COX-2 inhibitors can modulate apoptosis (51, 52), including in human colorectal epithelial tissues (53), and cancer stem cells may be more sensitive to NSAIDs-induced apoptosis relative to differentiated cells which is relevant to eradicating micrometastases (54).

From a clinical perspective, identifying the lowest dose of aspirin that can achieve anti-tumor effects while minimizing potential toxicities is critical. In a prior study, we reported that the 81 mg daily aspirin dose suppressed PGE_2 levels equally as did the 650 mg daily dosage in the colorectal mucosa of patients with prior adenomas (55). The anti-tumor benefits of aspirin are achieved with a trade-off of increased toxicities, as described in a meta-analysis of 22 randomized trials of aspirin for vascular disease prevention. Most notable are the risks of gastrointestinal toxicities, mainly ulcers and GI bleeding (RR=1.62; 95% CI, 1.25–2.09), or intracranial bleeding (RR=1.65; 95% CI, 1.06–5.99). In the meta-analysis, there was no difference in the rate of adverse events between patients receiving low dose (75–162.5 mg/day) vs standard dose(162.5–325 mg/day) aspirin (56). The risks *vs* potential benefits of aspirin must always be considered when advocating its use in patients. While aspirin is currently not recommended for patients at average risk of developing CRC or in patients with removal of prior adenomatous polyps, its use in high risk patients such as those with advanced adenomas or prior CRC may be warranted on an individualized basis. However, unresolved issues include the minimally effective dose, optimal duration, and the role of aspirin in patients already undergoing colonoscopic surveillance. For the adjuvant therapy of CRC, existing data justify the prospective evaluation of aspirin in this setting and a clinical trial (ASCOLT) is ongoing. Furthermore, the addition of celecoxib to standard chemotherapy with FOLFOX is being studied in an ongoing phase III adjuvant therapy trial (CALBG 80702).

Conclusions

More than a century after it was first synthesized, the therapeutic benefits of aspirin continue to emerge. Aspirin has been shown to protect against the recurrence of colorectal adenomas and carcinomas, and compelling evidence suggests its efficacy as an adjuvant agent in a molecular subset. Specifically, aspirin may selectively and potently inhibit colon cancer recurrence and improve survival in patient tumors with *PI3KCA* mutations. While this finding is compelling, the modest number of patients whose tumors carried *PIK3CA* mutations and who also used aspirin in these studies necessitates caution in their interpretation and underscores the need for prospective validation. Aspirin is currently being studied as adjuvant therapy in an ongoing trial in CRC patients (ASCOLT), and another adjuvant study evaluates the benefit of adding celecoxib to FOLFOX in node-positive colon cancer patients (CALGB-80702). In both trials, a comparison of survival based on *PIK3CA* mutation status will be performed and will yield further efficacy data. Prospective evaluation will be challenging due to the relatively small number of patients whose tumors carry the *PIK3Ca* mutation, and studies will also need to address the issue of duration of aspirin therapy needed to achieve clinical benefit. Research into the mechanistic basis of aspirin's efficacy in *PIK3CA* mutated CRCs is eagerly awaited. In an era of targeted therapy that is increasing health care costs, aspirin is an inexpensive and well tolerated drug that may prove to be an effective agent to prevent colon cancer recurrence.

Acknowledgments

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Translational relevance

Aspirin has been shown to reduce the incidence of colorectal cancer (CRC), and accumulating evidence suggests that aspirin may improve the clinical outcome of CRC patients following surgical resection. Recent data from large observational studies indicate that the survival benefit of aspirin may be confined to specific molecular subsets defined by *phosphatidylinositol 3-kinase (PI3KCA)* mutation status and cyclooxygenase-2 (COX-2) expression levels. Furthermore, regular aspirin intake was found to be associated with a reduced risk of developing CRCs with wild-type *BRAF* alleles, but not tumors with activating V600E point mutations. Together, these data suggest that aspirin may selectively exert its anti-tumor effects in specific molecular subsets, thereby identifying potential predictive biomarkers for aspirin efficacy in CRC patients.
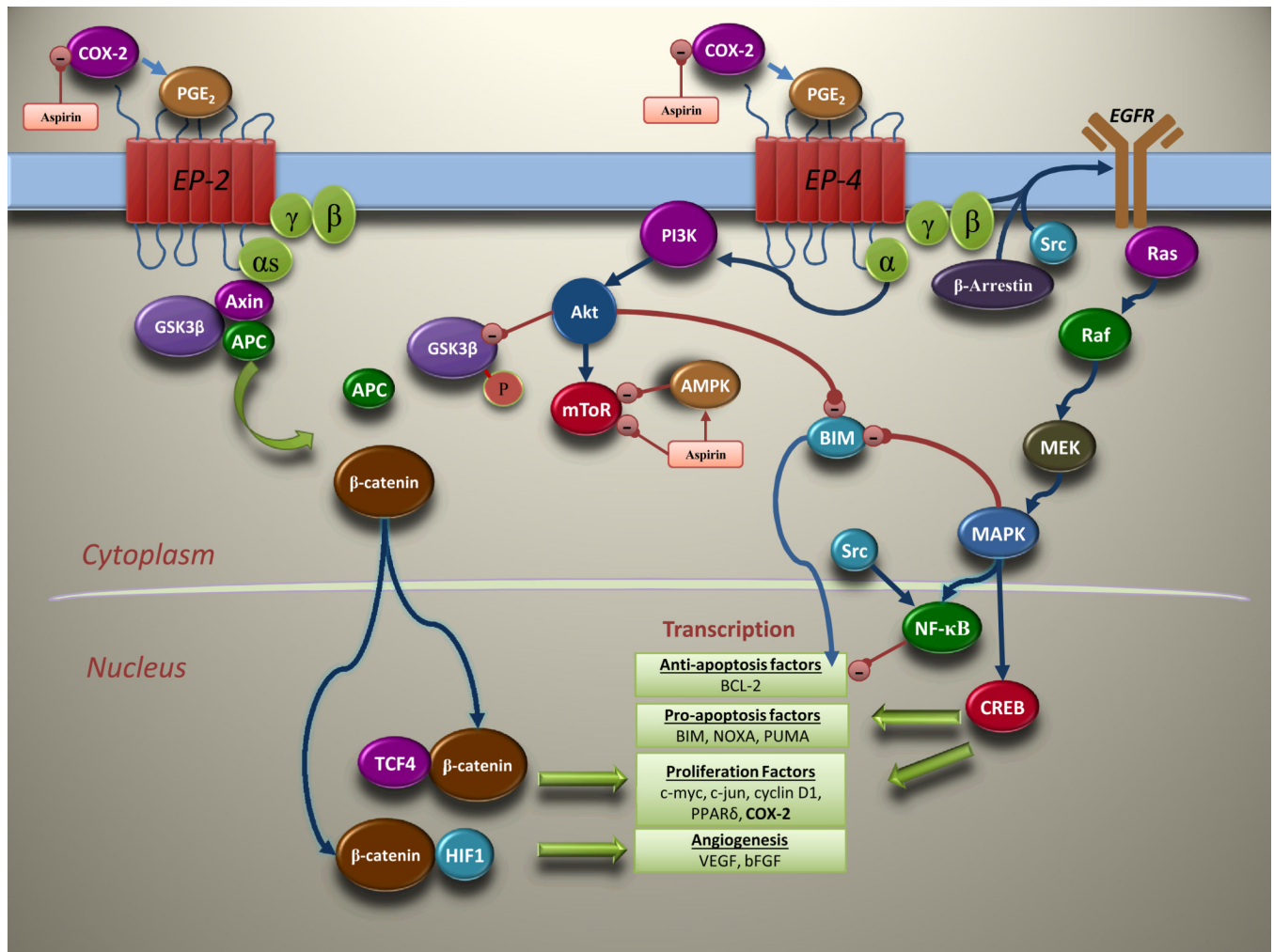
Figure 1.
Molecular pathways regulated by PGE$_2$ that are inhibited by aspirin. PGE$_2$ promotes cancer cell growth by binding to its EP receptors and modulating signaling pathways downstream of its receptors. In addition to binding Axin (58), the EP4 receptor activates PI3K which phosphorylates GSK-3β to promote β-catenin-mediated transcription (40). PGE$_2$ signaling is also implicated in c-Src and β-arrestin-mediated transactivation of EGFR and upregulation of the RAS-RAF-MAPK pathway (59).

Table 1

Biomarkers indicating aspirin efficacy in colorectal cancer

Biomarkers indicating aspirin efficacy in colorectal cancer				
Name of Study group	**Study population (N)**	**Biomarker under study**	**Aspirin dose used**	**Results [Multivariate HR (95% CI)]**
Biomarkers predicting survival in established CRC				
Nurses' Health and Health professional Follow-up study(7)	Stage I, II & III CRC (N=1279)	COX-2	325 mg	In COX-2 overexpressing tumors: HR 0.39 (0.20–0.76) for CRC-specific mortality In COX-2 negative tumors: HR 1.22 (0.36–4.18) for CRC-specific mortality
Nurses' Health and Health professional Follow-up study(37)	Stage I-IV CRC (N=964)	PIK3CA	325 mg	In PIK3CA-WT tumors: HR 0.93 (0.68–1.28) for CRC-specific mortality In PIK3CA mutant tumors: HR 0.18 (0.05–0.60) for CRC-specific mortality In PIK3CA-WT and COX-2 positive tumors: Stage-adjusted HR 0.97 (0.73–1.29) for overall survival In PIK3CA-mutant and COX-2 positive tumors[*] Stage-adjusted HR 0.34 (0.14–0.82) for overall survival
Biomarkers predicting CRC incidence				
Nurses' Health and Health professional Follow-up study(7)	NHS (N=82,911) HPFS (N=47,363) Stage I-IV CRC (N=636)	COX-2	325 mg	RR 0.64 (0.52 to 0.78) for incidence of COX-2 overexpressing tumors RR 0.96 (0.73 to 1.26) for incidence of COX-2 negative tumors
Nurses' Health and Health professional Follow-up study(22)	NHS (N=82095) HPFS (N=45770) Stage I-IV CRC (N=1226)	BRAF	325 mg	HR 1.03 (0.76–1.38) for incidence of BRAF-mutant CRC HR 0.73 (0.64–0.83) for incidence of BRAF-WT CRC Effect on BRAF-WT tumors based on COX-2 expression: HR 0.67 (0.56–0.81) for incidence of COX-2 overexpressing CRC HR 0.86 (0.67–1.09) for incidence of COX-2 negative CRC

[*] The study recorded zero deaths in the cohort which was both PIK3CA mutant and COX-2 expressing (n=23).

Abbreviations: CRC, colorectal cancer; HR ,Hazard ratio; COX-2, cyclooxygenase-2; WT, wild-type

Table 2

Aspirin adjuvant studies in patients with established colorectal cancer

Efficacy of aspirin in patients with surgically resected colorectal cancer				
Name of Study group	**Type**	**Study population (N)**	**Aspirin dose used**	**Result from aspirin use post-diagnosis [Multivariate HR (95% CI)]**
CALGB 89803(28)	Subgroup analysis in a randomized controlled trial	Stage III CRC (N=830)	325 mg	HR 0.48 (95% CI, 0.24–0.99) for disease free-survival HR 0.52 (95% CI, 0.19–1.46) for death (OS)
Nurses' Health and Health professional Follow-up study(27)	Prospective cohort study	Stage I, II & III CRC (N=1279)	325 mg	HR 0.71 (95% CI, 0.53–0.95) for CRC–specific mortality HR 0.79 (95% CI, 0.65–0.97) for overall mortality In aspirin non-users before diagnosis: HR 0.53 (95% CI, 0.33–0.86) for CRC–specific mortality In aspirin users before diagnosis: HR 0.89 (95% CI, 0.59–1.35) for CRC–specific survival
Eindhoven Cancer Registry and PHARMO prescription registry(30)	Retrospective cohort study	Stage I-IV CRC (N=4481)	80 mg	RR 0.77 (95% CI, 0.63–0.95) for CRC-specific mortality RR 0.65 (95% CI, 0.50–0.84) for colon cancer mortality RR 1.03 (95% CI, 0.75–1.40) for rectal cancer mortality
Health Informatics Centre Registry, Scotland(29)	Retrospective cohort study	Stage I-IV CRC (N= 2916)	75 mg	HR 0.67 (95% CI, 0.57–0.79) for CRC-specific mortality HR 0.72 (95% CI, 0.57–0.91) for colon cancer mortality HR 0.80 (95% CI, 0.58–1.11) for rectal cancer mortality

Abbreviations: CRC, colorectal cancer; HR, Hazard ratio; OS, overall survival; RR, relative risk.

Table 3

Efficacy of aspirin in secondary prevention of colorectal cancer

Efficacy of aspirin in secondary prevention of colorectal cancer				
Name of Study group	**Type**	**Study population (N)**	**Aspirin dose used**	**Result from aspirin use [Multivariate HR (95% CI)]**
Aspirin/Folate Polyp Prevention Study(14)	Randomized controlled trial	1121 patients with H/O colorectal adenomas	81 mg or 325 mg/ day	In the 81 mg patient group: Unadjusted RR 0.81 (0.69–0.96) for developing any adenoma. Adjusted RR 0.83 (0.70–0.98) for developing any adenoma. In the 325 mg patient group: Unadjusted RR 0.96 (0.81–1.13) for developing any adenoma. Adjusted RR 0.95 (0.80–1.12) for developing any adenoma.
APACC Trial(57)	Randomized controlled trial	272 patients with H/O colorectal adenomas	300-mg or 160 mg/ day	In the 160 mg group: RR 0.85 (95% CI, 0.57–1.26) for recurrent adenoma In the 300 mg group: RR 0.61 (95% CI, 0.37–0.99) for recurrent adenoma
Colorectal Adenoma Prevention Study(13)	Randomized controlled trial	635 patients with H/O colorectal cancer	325 mg/ day	Adjusted RR 0.65 (95% CI, 0.46–0.91) for any recurrent adenoma

Abbreviations: CRC, colorectal cancer; HR, Hazard ratio; RR, relative risk.