

OPINION

Aspirin and colorectal cancer: the promise of precision chemoprevention

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Abstract | Aspirin (acetylsalicylic acid) has become one of the most commonly used drugs, given its role as an analgesic, antipyretic and agent for cardiovascular prophylaxis. Several decades of research have provided considerable evidence demonstrating its potential for the prevention of cancer, particularly colorectal cancer. Broader clinical recommendations for aspirin-based chemoprevention strategies have recently been established; however, given the known hazards of long-term aspirin use, larger-scale adoption of an aspirin chemoprevention strategy is likely to require improved identification of individuals for whom the protective benefits outweigh the harms. Such a precision medicine approach may emerge through further clarification of aspirin's mechanism of action.

Despite greater adoption of population screening and considerable advances in understanding the molecular basis of colorectal neoplasia, colorectal cancer (CRC) remains the second leading cause of cancer deaths in the United States, with an estimated 129,700 new cases expected for 2015 (REF. 1). Aspirin (acetylsalicylic acid) has emerged as perhaps the most promising agent for the chemoprevention of CRC^{2,3}. This is due in large part to remarkably consistent data that have emerged from numerous basic, clinical and epidemiological studies over the past several decades (FIG. 1). The United States Preventive Services Task Force (USPSTF) originally recommended against the use of aspirin for the prevention of CRC in 2007 (REF. 4). However, in 2015, in their updated draft recommendations⁵ for low-dose aspirin in the primary prevention of cardiovascular disease (CVD), the USPSTF acknowledged that supporting evidence had become so compelling that CRC prevention warranted inclusion in their rationale for routine aspirin use among those aged between 50 and 69 with specific cardiovascular risk profiles^{6,7} (BOX 1). This decision distinguishes aspirin as the first pharmacological agent to be endorsed for cancer chemoprevention in a population not characterized as having a high risk of developing cancer. Nevertheless, the

USPSTF also cautioned against the potential harms associated with regular aspirin use and highlighted the need to clarify the mechanisms by which aspirin prevents the development of colorectal neoplasia.

In this Opinion article, we review the weight of the evidence supporting the chemopreventive potential of aspirin and highlight advances in our understanding of its mechanisms of action. We challenge the notion that aspirin prevents cancer through a single, dominant pathway and propose an integrative multi-pathway model for its mode of action. Furthermore, we highlight how these pathways can be leveraged to develop mechanistic biomarkers for personalized risk stratification. Such biomarkers may then be translated clinically in a precision medicine approach that is crucial to the future of aspirin chemoprevention.

Supporting evidence

The epidemiological evidence supporting the efficacy of aspirin for the prevention of cancer, especially CRC, is substantial^{2,3,8–11}. A recent pooled analysis of 10 cohort and case–control studies demonstrated that aspirin use was associated with a 29% reduction in the incidence of CRC (odds ratio (OR) = 0.71; 95% confidence interval (CI) 0.66–0.77)¹². More recently, a study from northern

Denmark reported a 27% reduction in CRC risk (OR = 0.73; 95% CI 0.54–0.99) in those who consecutively filled low-dose aspirin prescriptions for more than 5 years¹³. Beyond cohort and case–control studies, additional data have also emerged through secondary analyses of randomized controlled trials (RCTs) originally conducted to examine the role of aspirin in the prevention of CVD that have subsequently been linked to long-term cancer outcomes (TABLE 1). A meta-analysis of 3 such trials found that aspirin treatment for 5 or more years at doses of 75–300 mg per day reduced the long-term risk of CRC by 24% (hazard ratio (HR) = 0.75; 95% CI 0.56–0.97)¹⁴. Because aspirin treatment was randomized, these findings are less likely to be due to confounding effects associated with the reason for use, which may complicate the analysis of data from cohort or case–control studies.

Aspirin RCTs with cancer outcomes. Until recently, RCTs of aspirin that included cancer outcomes as predefined end points were limited in number and generated mostly discouraging findings. The Physicians' Health Study (PHS), a placebo-controlled RCT of alternate-day 325 mg aspirin among 22,071 male physicians, found no association between aspirin use and CRC incidence over 12 years of follow-up¹⁵. The Women's Health Study (WHS), the largest placebo-controlled RCT of aspirin for primary prevention, compared alternate-day 100 mg aspirin with a median duration of treatment of 9 years¹⁶. The results at completion of the originally planned follow-up of 10 years provided no evidence of an effect of aspirin on CRC incidence¹⁶. In addition, the Colorectal Adenoma/carcinoma Prevention Programme 2 (CAPP2) trial observed no reduction in the risk of colorectal neoplasia among carriers of Lynch syndrome, a hereditary CRC syndrome, after 2.5 years of planned follow-up in those assigned to 600 mg of aspirin per day¹⁷.

However, extended follow-up of RCTs has provided more promising evidence. For example, in the WHS, an inverse association between those randomized to aspirin and CRC incidence emerged 10 years after randomization (HR = 0.80; 95% CI 0.67–0.97)¹⁸. In the CAPP2 trial, a secondary preplanned analysis, conducted

Milestones and notable findings relating to aspirin and risk of CRC

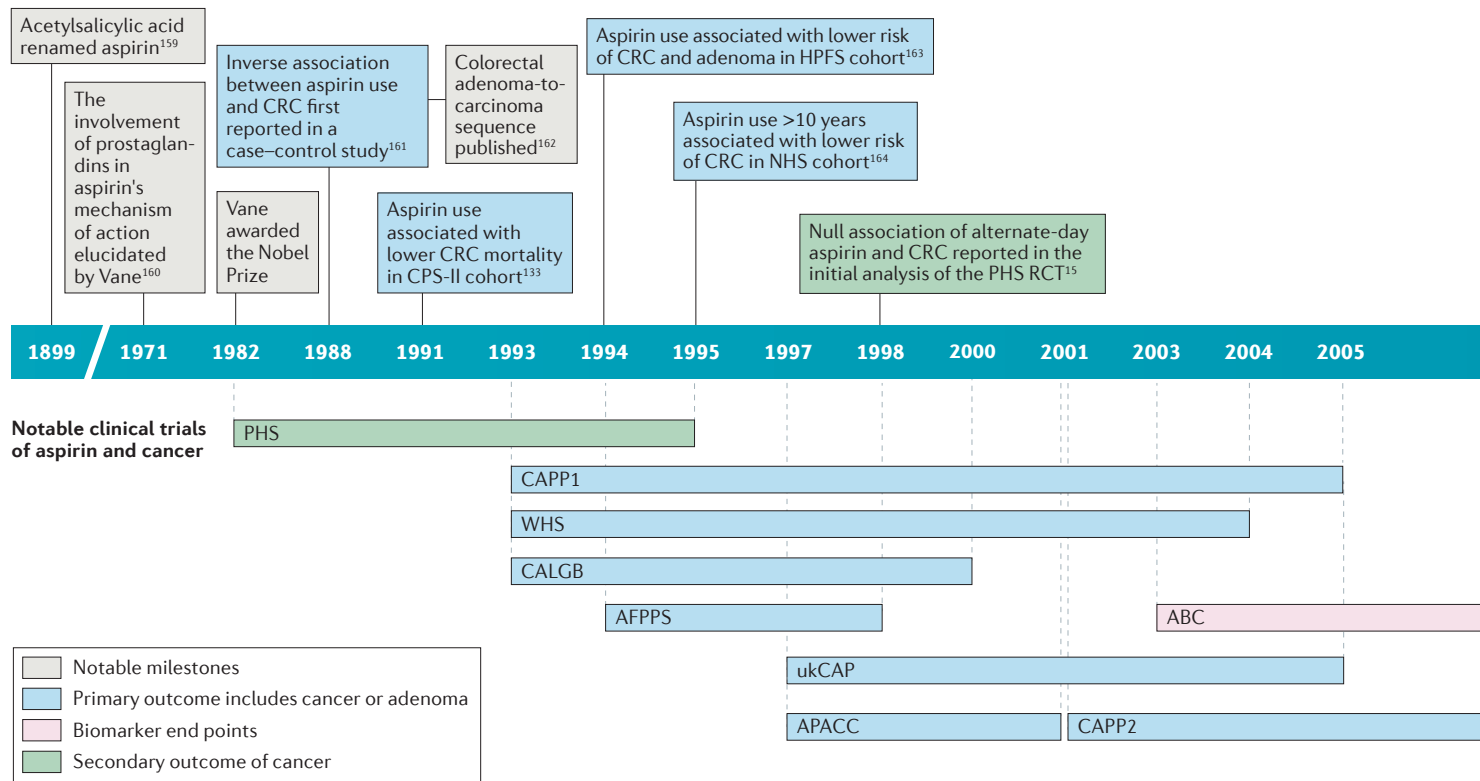


Figure 1 | **Timeline of notable human studies in aspirin chemoprevention.** Decades of research have substantially clarified the anticancer effect associated with the use of aspirin. Recent compelling human data have also influenced the United States Preventive Services Task Force (USPSTF) to support the use of aspirin for the primary prevention of colorectal cancer (CRC) in certain subgroups of the US population. Asterisks indicate trials that are ongoing and have an unknown end date. ABC, Aspirin and the Biology of the Colon; AFPPS, Aspirin/Folate Polyp Prevention Study; APACC, Association pour la Prévention par l'Aspirine du Cancer Colorectal; ARRIVE, Aspirin to Reduce Risk of Initial Vascular Events; ASCOLT, Aspirin for Dukes C and High Risk Dukes B Colorectal Cancers; ASPREE, Aspirin in Reducing Events in the Elderly; ASPIRED, ASPIrin Intervention for the REDuction of colorectal cancer risk; CALGB, Colorectal Adenoma prevention study originated in the cooperative trials group cancer and Leukaemia Group B; CAPP, Colorectal Adenoma/carcinoma Prevention Programme; CPS-II, Cancer Prevention Study II; CVD, cardiovascular disease; GxE, gene by environment; HPFS, Health Professionals Follow-up Study; HPGD, 15-hydroxyprostaglandin dehydrogenase; J-CAPP, Japan Colorectal Aspirin Polyps Prevention; J-FAPP, Japan Familial Adenomatous Polyposis Prevention; NHS, Nurses' Health Study; NSAID, non-steroidal anti-inflammatory drug; PGE-M, major metabolite of prostaglandin E₂; PHS, Physicians' Health Study; PIK3CA, PI3K catalytic subunit- α ; PTGS2, prostaglandin-endoperoxide synthase 2; RCT, randomized clinical trial; seAFood, Systematic Evaluation of Aspirin and Fish Oil; sTNFR2, soluble TNF receptor 2; ukCAP, United Kingdom Colorectal Adenoma Prevention; WHS, Women's Health Study.

after the first participant reached 10 years of follow-up, did show a significantly reduced risk of CRC in the per-protocol analysis (HR = 0.41; 95% CI 0.19–0.86) and after accounting for the multiple primary events commonly seen among patients with Lynch syndrome (incidence rate ratio (IRR) = 0.56; 95% CI 0.32–0.99)¹⁹. Nonetheless, these findings may not be generalizable to sporadic, non-hereditary CRC, as Lynch syndrome tumours arise through a distinct pathway of defective DNA mismatch repair^{20,21} and account for fewer than 3% of all cases of CRC.

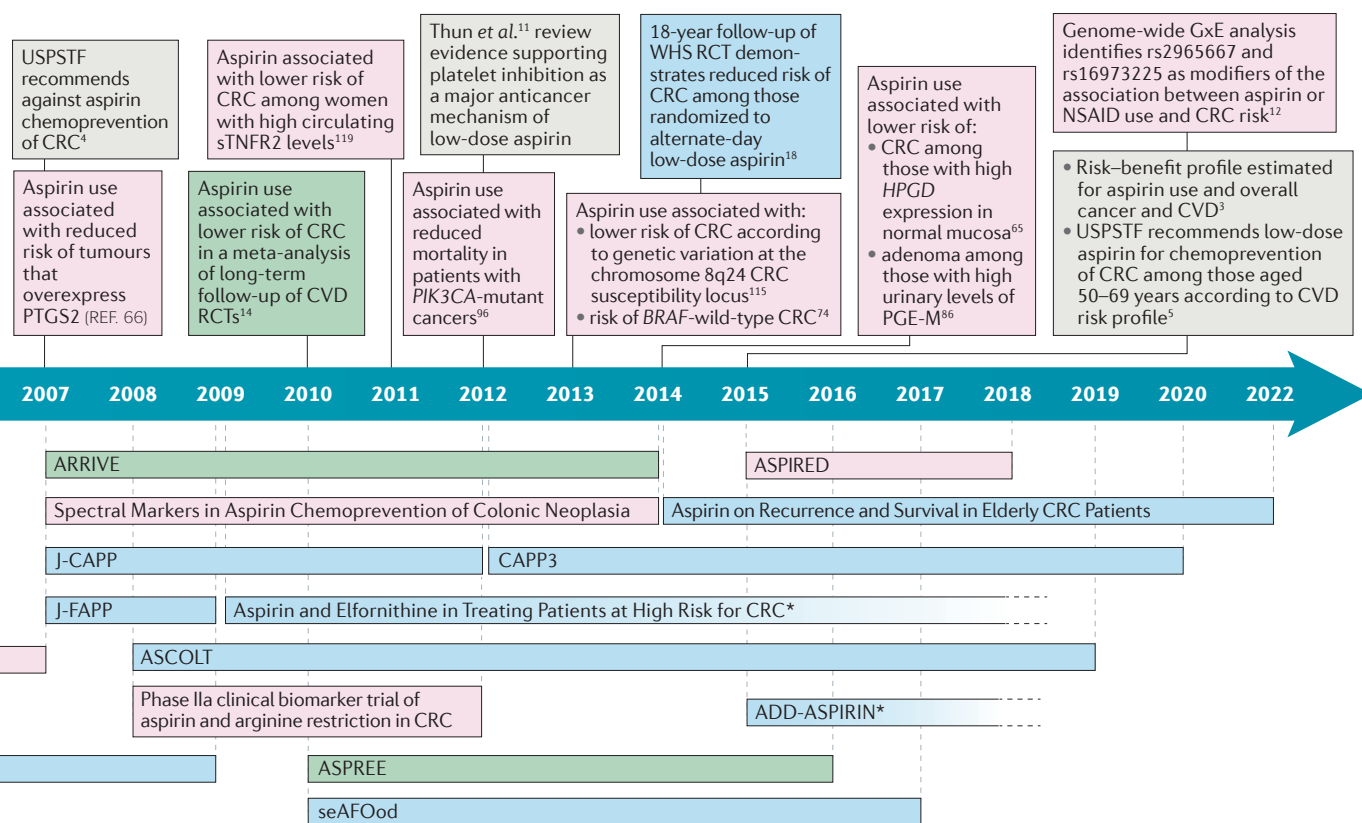
Thus, there has not yet been a definitive RCT investigating the effect of long-term daily aspirin treatment at a range of doses with sporadic CRC as a pre-specified end point over at least 10 years of defined follow-up. Such a trial has been challenging

to pursue owing to the large number of subjects and prolonged follow-up required for incident cancers to emerge. Moreover, the conduct of such an RCT would be complicated by problems of long-term compliance with an aspirin regimen, as well as the rising prevalence of aspirin use for CVD prevention, which leads to significant treatment-group crossover over time. The resulting decrease in assigned aspirin use in the treatment group and increase in non-trial aspirin use in the placebo group will dilute a potential randomized treatment effect.

Aspirin RCTs with precancerous outcomes.

In lieu of such studies, RCTs designed to examine the impact of aspirin on colorectal adenomas, the precursor to the majority of CRCs^{22–25}, have provided compelling

evidence of causality and the efficacy of aspirin chemoprevention (TABLE 2). Adenoma prevention trials are more feasible, given the relatively short-term follow-up needed to examine the recurrence of adenomas in individuals at higher risk for colorectal neoplasia. Four trials (namely, the Aspirin/Folate Polyp Prevention Study (AFPPS), Association pour la Prévention par l'Aspirine du Cancer Colorectal (APACC), Cancer and Leukaemia Group B (CALGB) and the United Kingdom Colorectal Adenoma Prevention (ukCAP) trial) report a range of reductions in the risk of recurrent adenoma (4–39%) among individuals with a high risk of sporadic CRC^{26–29}. Recently, the Japan Colorectal Aspirin Polyps Prevention (J-CAPP) trial showed that aspirin reduced the recurrence of any



adenoma or CRC (relative risk (RR) = 0.60; 95% CI 0.36–0.98)³⁰. Interestingly, in a secondary analysis, prevention of adenoma recurrence seemed to be restricted to non-smokers, providing evidence of a possible interaction with cigarette smoking. This interaction was recently corroborated by a large cross-sectional study of colorectal polyps³¹ and an RCT of a combined aspirin, calcitriol and calcium pill³². The RCT found no overall association between treatment and adenoma recurrence, but provided evidence of an interaction between treatment effects and smoking status³². Although the study encountered several challenges — including early trial termination, a non-traditional combination drug, high dropout rate and low compliance — the authors also attributed the null association to the relatively large proportion of smokers in the study population. As such, further investigation into lifestyle modifiers of aspirin chemoprevention, such as cigarette smoking, is warranted.

Ongoing aspirin RCTs. Over the coming years, additional ongoing RCTs (Aspirin to Reduce Risk of Initial Vascular Events (ARRIVE)³³, Aspirin for Dukes C and High Risk Dukes B Colorectal Cancers (ASCOLT)³⁴, ASPIRIN in Reducing Events in

the Elderly (ASPREE)³⁵, ASPIRIN Intervention for the REDuction of colorectal cancer risk (ASPIRED)³⁶, CAPP3 (REF. 37) and Systematic Evaluation of Aspirin and Fish Oil (seAFOod) polyp prevention trial³⁸), combined with continued collection of long-term outcome data from completed trials, are expected to add to the growing evidence in support of aspirin chemoprevention of CRC. All things considered, the ‘perfect’ trial of aspirin and CRC prevention may not be feasible. However, the consistency of both epidemiological and RCT evidence thus far provides a compelling basis from which to more broadly consider the use of aspirin for chemoprevention, especially for CRC, a consensus opinion that is shared by many authorities in the field^{2,3,8,9,11}. Indeed, the current level of ‘imperfect’ evidence was considered sufficiently persuasive to lead the USPSTF to incorporate the effect of aspirin on CRC in its broader recommendation for adults in the United States between the ages of 50 and 69 with a greater than 10% 10-year risk of CVD to consider chronic disease prophylaxis with low-dose aspirin³⁹.

Assessing risk–benefit for chronic aspirin use. BOX 2 presents a summary of the potential side effects associated with regular aspirin use. Several studies have estimated the magnitude of such risks, particularly

gastrointestinal bleeding, through assessment of the severe adverse events reported during RCTs. A meta-analysis of 35 RCTs using 75–325 mg daily doses of aspirin alone estimated an HR for major gastrointestinal bleeding of 1.31 (95% CI 1.21–1.42)⁴⁰. For average-risk individuals (without a history of bleeding and not taking other anticoagulant or antiplatelet medications), this translates into one or two gastrointestinal bleeding events per 1,000 person-years. Most⁴¹, but not all^{42,43}, studies find that such toxicities are largely dose related, with the hazards for bleeding being generally higher in patients treated with 300–325 mg than in those treated with 75–162.5 mg^{44–47}. However, in 2012, a meta-analysis of RCTs by Rothwell and colleagues¹⁰ found that major extracranial bleeding (mainly gastrointestinal) associated with aspirin occurred primarily in the short term (<3 years) following initiation of aspirin use, with likelihood increasing with age. They concluded that in the long term (≥3 years), low-dose (<300 mg) aspirin use was not significantly associated with the risk of such events¹⁰. Nonetheless, the consideration of such risks highlights the importance of developing a precision medicine strategy to identify those individuals who are most likely to benefit from a prophylactic aspirin regimen.

Molecular risk stratification

The first efforts to develop precision medicine approaches to improve the therapeutic index for aspirin chemoprevention focused on pharmacogenomics⁴⁸, and particularly on variants of genes encoding the highly polymorphic CYP2C9 (cytochrome p450 family 2 subfamily C polypeptide 9) and UGT (UDP-glucuronosyltransferase) enzymes, which are responsible for the metabolism of aspirin and non-steroidal anti-inflammatory drugs (NSAIDs). However, data have not been consistent: most^{49–55}, but not all^{56,57}, recent studies have demonstrated no significant modification of aspirin or NSAID responsiveness according to UGT or CYP genotype. An additional precision medicine approach to aspirin chemoprevention

may emerge through advances in our understanding of the associated anticancer mechanisms. These biological pathways may be exploited for molecular biomarkers to stratify risk–benefit (TABLE 3). In this section, we review the progress made thus far in elucidating aspirin’s mode of action, which has been facilitated by advances in biobanking, genomics, metabolomics and integrative molecular epidemiology over the past decade.

Prostaglandin synthesis and catabolism. The capacity of aspirin to irreversibly bind and acetylate, and therefore inhibit, prostaglandin-endoperoxide synthase 1 (PTGS1; also known as COX1) and PTGS2 (also known as COX2) has been widely considered to be central to

its chemopreventive mechanisms^{58–66} (FIG. 2). These enzymes perpetuate pro-inflammatory signals, which promote cellular proliferation, angiogenesis and apoptotic resistance. PTGS enzymes are responsible for the conversion of arachidonic acid into downstream effectors that are further metabolized into prostaglandins (PGs) and related eicosanoids (such as PGE₂, PGD₂, PGF₂, thromboxane A₂ (TXA₂) and prostacyclin; collectively known as prostanoids). PGs can then bind to a number of downstream targets, including plasma membrane and nuclear receptors.

Increased synthesis of PGE₂, which is directly attributable to PTGS2 activity, has been consistently observed in patients with colorectal neoplasia and has been shown to promote colorectal carcinogenesis^{67,68}. Genetic deletion of *Ptgs2* or any of several PGE₂ receptor genes results in decreased intestinal tumour incidence and burden in mouse models of CRC^{69–73}. The relevance of this chemopreventive mechanism in humans is further supported by molecular epidemiology studies. Within two large cohorts, regular aspirin use conferred a significant reduction in the risk of cancers that overexpress PTGS2 (RR=0.64; 95% CI 0.52–0.78) as measured by immunohistochemical staining of the tumour⁶⁶. However, aspirin use was not associated with the risk of cancers with low or negative PTGS2 expression (RR=0.96; 95% CI 0.73–1.26; $P_{\text{heterogeneity}}=0.02$). More recently, these findings were extended by examining the effect of aspirin on other molecular subtypes, including CRCs with mutations in *BRAF*, a proto-oncogene in the MAPK signalling cascade⁷⁴. Aspirin use was associated with lower risk of *BRAF*-wild-type CRC (HR=0.73; 95% CI 0.64–0.83) but not *BRAF*-mutant CRC (HR=1.03; 95% CI 0.76–1.38); $P_{\text{heterogeneity}}=0.005$). In particular, the benefit of aspirin seemed to be most evident among cancers that overexpress PTGS2 and were *BRAF* wild-type. Because *BRAF* mutation is strongly associated with cancers that seem to emerge through epigenetic dysregulation and thus the serrated carcinogenesis pathway^{75–77}, these results suggest that those cancers with upregulated MAPK signalling, and perhaps serrated cancers, may be less dependent on PTGS2 or PG-mediated pathways for their growth.

15-hydroxyprostaglandin dehydrogenase (HPGD; also known as 15-PGDH), the primary enzyme that catabolizes PGs, functions as a metabolic antagonist, or ‘brake’, for PTGS2. *In vivo* experiments have demonstrated that *Hpgd*-null mice are more susceptible to colon tumorigenesis⁷⁸ and are insensitive

Glossary

Case–control studies

A type of observational study design in which two groups differ according to outcome (for example, in which those with the disease or condition (cases) are compared with disease-free individuals (controls)). These studies can be nested within a larger cohort with only a subset of the larger control population being used.

Confidence interval

(CI). A measure of the uncertainty associated with a sample estimate for a given parameter.

Events per 1,000 person-years

Also known as the incidence density rate or person–time incidence rate. This approach normalizes event observations to the amount of observation time and is useful if observation times are not constant across a sample population or the risk of an event varies over time.

Fractal dimension

(FRAC). A spectroscopic measure of light scattering due to nanoscale architectural changes in mass density of cellular components that has been associated with early neoplastic changes in epithelial cells.

Glucuronidation

The addition of glucuronic acid to a substrate.

H2 blocker

A drug that inhibits the production of gastric acid by targeting histamine H2 receptors of gastric parietal cells.

Hazard ratio

(HR). A measure of the ratio of the hazard rates (the rate at which an event occurs) for a given outcome (for example, cancer) described by an explanatory variable (for example, aspirin versus placebo).

Incidence rate ratio

(IRR). A measure of the ratio between the rates of how often an outcome (for example, cancer) occurs in a population at any given time according to an explanatory variable (for example, aspirin versus placebo).

Odds ratio

(OR). A measure of association representing the odds (the probability of disease divided by 1 minus the probability

of an outcome according to an explanatory variable (for example, aspirin users versus non-users).

$P_{\text{heterogeneity}}$

A statistic that measures the significance of the difference (or heterogeneity) between two effect sizes.

$P_{\text{interaction}}$

A statistic that measures the significance of the effect of a given exposure or explanatory variable on a second exposure or explanatory variable, and vice versa.

Proton pump inhibitor

A drug that inhibits the production of gastric acid by targeting the proton pump transport activity of gastric parietal cells. Proton pump inhibitors are generally more effective than H2 blockers.

P_{trend}

A statistic that measures the significance of a correlation of effect size, either positive or negative, across a continuous or ordinal variable.

Relative risk

(RR). The probability of an outcome (for example, cancer) occurring in one group (for example, aspirin) versus the probability in a comparison group (for example, placebo).

Serrated carcinogenesis pathway

A carcinogenesis pathway in which colorectal tumours are characterized by epigenetic dysregulation (promoter hypermethylation), *BRAF* mutation and activation, and often microsatellite instability, rather than adenomatous polyposis coli (APC) mutations and chromosomal instability.

Single nucleotide polymorphism

(SNP). A common type of genetic variation in which a single nucleotide or base occurs at a specific position in the genome that is different from the expected or reference nucleotide.

Spectral slope

(SPEC). A spectroscopic measure of light scattering due to nanoscale architectural changes in the size distribution of cellular components that has been associated with early neoplastic changes in epithelial cells.

to NSAID-based chemoprevention⁷⁹. The Adenoma Prevention with Celecoxib RCT provided human evidence that supported the role of HPGD as a crucial mediator of NSAID-based chemoprevention⁷⁹. Celecoxib, a selective PTGS2 inhibitor, prevented adenoma recurrence only in patients with above-average expression of *HPGD* transcripts in normal colonic mucosa, suggesting a cooperative role for HPGD and PTGS2 inhibition in reducing the risk of neoplasia. These provocative findings were extended to aspirin in a study of *HPGD* expression in the adjacent normal mucosa of CRC tumours⁶⁵. Aspirin use was associated with a reduced risk of CRC in those individuals with high expression of *HPGD* in normal colon tissue (HR = 0.49; 95% CI 0.34–0.71). Conversely, in individuals with low colonic expression of *HPGD* there was no protection associated with aspirin use ($P_{\text{heterogeneity}} = 0.02$). These results highlight the potential importance of HPGD in conferring sensitivity to aspirin

and suggest that normal tissue markers such as *HPGD* mRNA may serve as predictive biomarkers for chemoprevention. If validated in other populations, a clinical strategy could include concurrent biopsies of normal mucosa among individuals who undergo resection of an adenoma during endoscopy. Assessment of the level of expression of *HPGD* in these biopsies could then be used to predict the likelihood of a response to aspirin administered for prevention of recurrent neoplasia.

Although germline mutations in *HPGD* have not been consistently associated with carcinogenesis in humans, a recent study demonstrated that alterations in *SLCO2A1*, the gene that encodes the PG transporter responsible for bringing substrate to HPGD, was associated with marked early-onset colonic neoplasia⁸⁰. Carriers of this mutation mimic the *Hpgd*-null mouse phenotype, demonstrating elevated systemic PGE₂ (as measured by higher levels of the major urinary metabolite of PGE₂,

known as PGE-M (11 α -hydroxy-9,15-dioxo-2,3,4,5-tetranor-prostane-1,20-dioic acid)⁸¹) and resistance to NSAID-based stabilization of PG synthesis⁸⁰. These results further support the role of disordered PG catabolism in carcinogenesis and suggest that *SLCO2A1* may be a potential genetic biomarker for chemoprevention.

Additional evidence of the relevance of PG balance in colorectal neoplasia has been obtained through the application of methods to quantify PGE-M. Urinary PGE-M is widely accepted as the most accurate quantification of *in vivo* systemic PGE₂ levels⁸². Multiple studies have demonstrated an increased risk of CRC and adenoma in individuals with higher pre-diagnostic urinary PGE-M levels^{83–85}. In addition to supporting the importance of PGs in carcinogenesis, urinary PGE-M levels may also have potential for molecular risk stratification. In the Nurses' Health Study (NHS), regular use of aspirin, and/or other NSAIDs, was associated with a 39%

Box 1 | Summary of the United States Preventive Services Task Force draft recommendation

In October 2015, the United States Preventive Services Task Force (USPSTF) released a draft statement⁵ recommending the use of aspirin for the primary prevention of colorectal cancer (CRC) and cardiovascular disease (CVD). This statement was based on the findings of an evidence review commissioned by the Agency for Healthcare Research and Quality for the USPSTF⁷ (see the table). The recommendation has received a B grade for adults aged 50–59 years with a 10% or greater 10-year CVD risk, who are not at increased risk for bleeding, have a life expectancy of at least 10 years and are willing to take aspirin for at least 10 years⁵. For adults aged 60–69 years with similar risk profiles, the recommendation was assigned a C grade⁵. A B grade indicates that there is “high or moderate certainty that the net benefit is moderate to substantial” and it is suggested that practitioners offer or provide this service⁵. A C grade indicates that there is “at least moderate certainty that the net benefit is small” and it is suggested that practitioners offer or provide the service to certain patients depending on individual circumstances⁵. Table adapted from REF. 7 with permission from the Agency for Healthcare Research and Quality. Updated information is available on the [USPSTF](#) website.

Age (years)	Gender	CVD risk (%) [*]	Aspirin benefits (prevented events per 1,000 people)			Aspirin harms (incurred events per 1,000 people)		Net balance (benefits versus harms)		
			Non-fatal CVD events [‡]	CVD deaths	CRC cases	GI bleeds [§]	Haemorrhagic stroke [§]	Net life-years gained	Quality-adjusted life-years gained	Net events prevented [¶]
50–59	Male	10	37.2	4.1	13.9	28.4	2.3	33.3	58.8	24.5
		15	43.4	5.4	12.1	26.0	2.8	39.5	64.4	32.2
		20	46.2	5.5	12.2	24.8	2.1	60.5	83.4	37.0
	Female	10	35.8	3.9	13.9	20.9	3.5	21.9	62.1	29.3
		15	36.7	4.7	13.5	20.0	3.4	33.4	71.6	31.5
		20	36.6	4.4	13.2	18.4	2.9	46.3	83.3	33.1
60–69	Male	10	26.6	3.3	11.2	31.4	3.1	–2.0	18.0	6.7
		15	32.2	4.0	10.4	29.8	2.4	9.6	30.9	14.3
		20	34.2	4.5	9.1	26.7	2.7	11.6	31.8	18.4
	Female	10	26.7	3.1	10.5	23.0	3.2	–1.2	28.4	14.1
		15	29.7	3.9	9.3	21.6	3.4	1.7	32.4	18.0
		20	30.3	4.4	9.7	21.7	3.3	4.8	36	19.4

^{*}Baseline 10-year CVD risk was estimated using the [American College of Cardiology and American Heart Association risk calculator](#). [‡]Non-fatal CVD events combine myocardial infarction and ischaemic strokes with congestive heart failure. [§]Gastrointestinal (GI) bleeds and haemorrhagic stroke include both fatal and non-fatal events. ^{||}Per 1,000 persons, as calculated using a microsimulation model for net benefits. Quality-adjusted life-years are a measure of the value of the quality (disease burden) and quantity of years lived for a certain health outcome. [¶]Net events prevented = (non-fatal CVD events + CVD deaths + CRC cases) – (GI bleeds + haemorrhagic stroke).

Table 1 | Placebo-controlled clinical trials of aspirin in the prevention of colorectal cancer

Trial name	Primary end point	Inclusion criteria	Aspirin dose	Participants (n)	Median duration of treatment (years)	RR (95% CI)	
						CRC incidence	CRC mortality
TPT ¹⁴	CVD	Men aged 45–69 years without a current or recent history of possible peptic ulceration, a history of possible or definite MI or stroke or other medication incompatible with trial treatment	75 mg per day	5,085	6.9	0.75 (0.56–0.97), pooled with UK-TIA and SALT*	0.61 (0.43–0.87), pooled with UK-TIA and SALT
UK-TIA ^{14*}	CVD	Those aged ≥40 years whose last cerebrovascular event had occurred >3 months earlier; who have had a disabling major stroke; patients with potential cardiac sources of embolism who were not taking anticoagulants	300 or 1,200 mg per day	2,449	4.4	See pooled results above*	See pooled results above
SALT ¹⁴	CVD	Those aged 50–79 years who had had a transient ischaemic attack (including amaurosis fugax), minor ischaemic stroke or retinal artery occlusion in the previous 3 months	75 mg per day	1,360	2.7	See pooled results above*	See pooled results above
BDT [†]	CVD	Male physicians without peptic ulcer, stroke or definite MI	500 mg per day	4,959	6.0	0.70 (0.51–0.97) ⁹	0.73 (0.49–1.10) ¹⁴
PHS ¹⁵	CVD and cancer	Male physicians aged 40–84 years who did not regularly use aspirin or other NSAID and did not have a history of MI, stroke, cancer, liver or renal disease, gout, peptic ulcer or contraindications to aspirin	325 mg every other day [§]	22,071	5.0	1.03 (0.83–1.28)	Not reported
WHS ¹⁸	CVD and cancer	Women, aged 45 years or older, with no history of cancer (except for non-melanoma skin cancer), CVD or other major chronic illness	100 mg every other day	39,876	9.0	0.80 (0.67–0.97)	Not reported
CAPP2 (REF. 19)	CRC	Carriers of Lynch syndrome	600 mg per day [§]	861	2.1	0.63 (0.35–1.13)	Not reported

BDT, British Doctors Aspirin Trial; CAPP2, Colorectal Adenoma/carcinoma Prevention Programme 2; CI, confidence interval; CRC, colorectal cancer; CVD, cardiovascular disease; MI, myocardial infarction; NSAID, non-steroidal anti-inflammatory drug; PHS, Physicians' Health Study; RR, relative risk; SALT, Swedish Aspirin Low Dose Trial; TPT, Thrombosis Prevention Trial; UK-TIA, UK-Transient Ischaemic Attack; WHS, Women's Health Study. *For aspirin doses of 75–300 mg, from Rothwell et al.¹⁴. †BDT was not placebo controlled. Comparisons with a 'no aspirin' control group. §Trials had a 2 × 2 factorial design. PHS: aspirin with or without β-carotene (50 mg per day). CAPP2: aspirin with or without resistant starch (30 g per day).

reduction in adenoma risk among women with high baseline PGE-M levels but not among those with low PGE-M levels⁸⁶. However, these results were not confirmed by a similar study performed within the AFPPS RCT, which concluded that aspirin chemoprevention of adenoma recurrence was independent of prostanoid levels⁸⁷. Thus, further studies are needed to determine the potential use of urinary PGE-M as a biomarker for chemoprevention.

Additional PG pathway-based approaches for precision chemoprevention have been discussed in previous reviews^{48,88}. For example, several gene-by-environment (GxE) interaction analyses have been pursued that focus on genetic variants associated with PG or aspirin function^{54,89–93}; however, few of these studies have demonstrated a significant interaction between aspirin use and genetic variants in the context of CRC or adenoma risk. One cohort study did observe

that the GA genotype of the single nucleotide polymorphism (SNP) rs2920421, which lies in an intronic region of *ALOX12* (which encodes arachidonate 12-lipoxygenase), was associated with a lower risk of CRC only among current NSAID users and not among never users or former users⁹⁴. This finding requires corroboration in other populations as candidate-gene-based approaches to GxE interaction studies have a high potential for false-positive results.

GxE interaction analyses in the context of genome-wide association studies (GWASs) have provided an important and complementary approach to candidate gene studies, facilitating the discovery of novel genome-wide significant interactions between germline variants and regular aspirin use with risk of CRC. A recent study of 8,634 CRC cases and 8,553 controls from 10 studies identified that the SNP rs2965667 (minor allele frequency (MAF) = 1.7%)

seems to predict a differential response to aspirin use with putative, albeit somewhat indirect, relevance to PG synthesis¹². For rs2965667, the use of aspirin and NSAIDs was associated with a lower risk of CRC among individuals with the TT genotype (OR = 0.66; 95% CI 0.61–0.70; $P = 7.7 \times 10^{-33}$). By contrast, aspirin use among those with either of the rare TA and AA genotypes (present in only 4% of individuals studied) was associated with an increased risk of CRC (OR = 1.89; 95% CI 1.27–2.81; $P = 0.002$). This SNP lies proximal to several candidate genes that encode proteins associated with the putative mechanisms of action of aspirin, which may be relevant to the association of aspirin with CRC survival^{95,96}. These genes include: microsomal glutathione S-transferase 1 (encoded by *MGST1*), which is a member of the membrane-associated proteins in eicosanoid and glutathione metabolism (MAPEG) family, is upregulated

in several cancers, including CRC, and has high sequence homology with PGE synthase 1 (encoded by *PTGES*; also known as *MGST1L1*)^{97–99}; LIM domain only 3 (*LMO3*), which is a known oncogene for other non-colorectal cancers^{100,101}; and PI3K catalytic subunit 2 γ (encoded by *PIK3C2G*), which is part of the PI3K family. Although these findings are promising, it is important to note that a limitation of the discovery-based GxE interaction approach using GWAS data is the stringent criteria required to achieve statistical significance at a genome-wide level and the possibility that genetic variation in key candidate pathways (such as the PG synthesis pathway) may not be well represented on current GWAS platforms. Thus, a genome-wide GxE interaction approach is associated with a greater likelihood of false-negative results.

Combined, these studies demonstrate multiple associations implicating modulation of PG synthesis as a major mode of action for aspirin chemoprevention and the potential of tissue, urinary and genomic biomarkers associated with PG synthesis for personalized risk stratification.

WNT- β -catenin signalling. Given the crucial importance of WNT dysregulation in the development of the majority of CRCs^{21,23}, the possible effects of aspirin on this signalling pathway have become a focus of mechanistic investigation. Briefly, this

pathway is activated through extracellular WNT ligand binding to surface receptors, which results in cytosolic stabilization of β -catenin and, ultimately, translocation of β -catenin to the nucleus¹⁰² (FIG. 2). In the nucleus, β -catenin binds to transcription factor 7 like-2 (encoded by *TCF7L2*) to form a transcriptional activation complex that leads to the expression of genes affecting cell proliferation and migration. In the absence of ligand, β -catenin becomes ubiquitinated and targeted for degradation through the formation of the destruction complex, which contains axin, adenomatous polyposis coli (APC), β -catenin and glycogen synthase kinase 3 β (GSK3 β). Aberrant activation of WNT signalling through APC loss-of-function results in cytosolic and nuclear accumulation of β -catenin and is observed in the majority of CRCs²¹. Aspirin treatment of human CRC cell lines reduces the cytoplasmic pool of β -catenin through inactivation of protein phosphatase 2A (PP2A), which is responsible for dephosphorylating the β -catenin amino acid residues (T41 and S45) that target β -catenin for ubiquitylation¹⁰³.

Mechanistic investigation into the potential for interaction of PGE₂ and WNT signalling has further highlighted the relevance of WNT modulation to aspirin chemoprevention, which has been previously reviewed in detail¹⁰⁴. In brief, PGE₂ seems to enhance WNT signalling via multiple

downstream effectors. Early evidence from *in vitro* cancer cell models demonstrated that PGE₂ stimulates tumour cell growth by binding to its plasma membrane receptor, EP2, thereby activating WNT- β -catenin signalling, in addition to PI3K and AKT signalling pathways¹⁰⁵. EP2 binds to axin, displacing it from its association with GSK3 β and thereby liberating β -catenin. The activation of PI3K and AKT contributes to β -catenin stabilization through direct phosphorylation and inactivation of GSK3 β . More recent evidence from developmental stem cell biology further supports the mechanistic role of PGE₂ in β -catenin stabilization through activation of cAMP and protein kinase A (PKA) signalling, which leads to additional inactivation of the destruction complex¹⁰⁶. Furthermore, PGE₂ increases transcription of *PPARD* (which encodes peroxisome proliferator-activated receptor- δ (PPAR δ)), which is a direct target gene of WNT signalling via the β -catenin-TCF7L2 transcriptional complex¹⁰⁷. PPAR δ is upregulated in some CRCs^{107,108} and has been associated with apoptotic resistance in CRC cell lines¹⁰⁹. Moreover, genetic deletion of *Ppard* in *Apc*^{Min/+} mouse models of CRC tumorigenesis abrogates the pro-tumorigenic effects of PGE₂ (REF. 110). Importantly, in *Apc*^{Min/+} mice with functional PPAR δ , aspirin derivatives reduced both intestinal tumorigenesis and tumour expression of PPAR δ ¹¹¹.

Table 2 | Placebo-controlled clinical trials of aspirin in the prevention of colorectal adenoma

Trial name	Primary end point	Inclusion criteria	Participants (n)	Median duration of follow-up (years)	Aspirin dose	RR (95% CI)		Refs
						Adenoma	Advanced adenoma	
AFPPS	Recurrent adenoma	Recent resection of colorectal adenomas	1,084	3.0	81 mg per day	0.81 (0.69–0.96)	0.62 (0.39–0.97)	165
					325 mg per day	0.96 (0.82–1.12)	0.86 (0.58–1.30)	
					Any aspirin	0.88 (0.77–1.02)	0.74 (0.52–1.06)	
APACC	Recurrent adenoma	Recent resection of colorectal adenomas	184	4.0	160 mg per day	0.88 (0.65–1.19)	1.19 (0.65–2.21)	165
					300 mg per day	1.03 (0.77–1.37)	0.58 (0.25–1.37)	
					Any aspirin	0.95 (0.75–1.21)	0.91 (0.51–1.60)	
CALGB	Adenoma	Recent resection of Dukes' stage A or B1 CRC or B2 CRC and 5-year disease-free survival	517	1.1	325 mg per day	0.61 (0.44–0.86)	0.77 (0.29–2.05)	165
ukCAP	Recurrent adenoma	Recent resection of colorectal adenomas	853	3.0	300 mg per day*	0.79 (0.63–0.99)	0.63 (0.43–0.91)	165
J-CAPP	Recurrent adenoma and CRC	Recent resection of colorectal adenomas and CRCs	311	2.0	100 mg per day	0.60 (0.36–0.98), pooled with advanced melanoma		30

AFPPS, Aspirin/Folate Polyp Prevention Study; APACC, Association pour la Prévention par l'Aspirine du Cancer Colorectal; CALGB, Colorectal Adenoma prevention study originated in the cooperative trials group cancer and Leukaemia Group B; CI, confidence interval; CRC, colorectal cancer; J-CAPP, Japan Colorectal Aspirin Polyps Prevention; RR, relative risk; ukCAP, United Kingdom Colorectal Adenoma Prevention. *Trial had a 2 × 2 factorial design. ukCAP: aspirin with or without folate supplement (0.5 mg per day).

Box 2 | Adverse events associated with aspirin use

In the United States, aspirin is widely available without a prescription. The most commonly reported adverse effects of aspirin use include nonspecific gastrointestinal (GI) symptoms, such as abdominal pain, dyspepsia, and nausea and vomiting¹⁵⁷. The most common serious adverse event is GI bleeding³⁹, with aspirin use associated with an odds ratio (OR) for GI bleeding of 1.59 (95% confidence interval (CI) 1.32–1.91) and an increase of 0.29 events per 1,000 person-years of aspirin exposure. Aspirin is also associated with intracranial bleeding (OR=1.27; 95% CI 0.98–1.66), although the absolute risk increase is lower (0.1 events per 1,000 person-years of aspirin exposure) than the risk of GI bleeding. Bleeding events seem to be correlated with dose and increasing age. The estimated adjusted incidence rate ratio for major bleeding events with each increasing year of age is 1.05 (95% CI 1.05–1.05). For GI symptoms and prophylaxis for bleeding, clinical management may include co-administration of a proton pump inhibitor or H₂ blocker¹⁵⁸.

People with the following conditions are generally advised against using aspirin³⁹: aspirin allergy or intolerance; active peptic ulcer; bleeding disorders; recent history of GI or intracranial bleeding; renal failure; severe liver disease; or low platelet count (thrombocytopenia). Individuals concurrently using anticoagulants or non-steroidal anti-inflammatory drugs (NSAIDs) may also be cautioned against using aspirin as these may increase the incidence or severity of bleeding events.

Beyond experimental models, mechanistically based candidate gene studies have provided proof of principle that aspirin may influence WNT signalling in humans. GWASs of CRC susceptibility have consistently identified a SNP, rs6983267, on chromosome 8q24 as an important risk locus^{112,113}; the T allele of rs6983267 was associated with a ~17% reduction in CRC risk. This SNP seems to have a role in WNT signalling by modulating the binding of TCF7L2, which leads to lower expression of the *MYC* proto-oncogene¹¹⁴. As experimental studies have demonstrated an effect of aspirin on WNT signalling, it follows that genetic variation at this locus may differentially influence aspirin function. Among a nested case–control study, the association of regular aspirin use with CRC differed significantly according to the genotype of this SNP¹¹⁵, with the benefit of aspirin predominantly evident among individuals with at least one T allele ($P_{\text{interaction}} = 0.01$). Compared with non-use, aspirin use was associated with ORs of 0.61 (95% CI 0.47–0.79) among those with the GT genotype and 0.52 (95% CI 0.35–0.78) among those with the TT genotype. By contrast, regular aspirin use was not associated with lower risk among individuals with the GG genotype (OR=0.99; 95% CI 0.70–1.40). Importantly, the T allele was also associated with decreased *MYC* expression in CRC tissue ($P_{\text{trend}} = 0.03$), and among those individuals with at least one protective T allele, aspirin use was specifically associated with lower risk of CRCs positive for nuclear accumulation of β -catenin (OR=0.44; 95% CI 0.26–0.75; $P_{\text{interaction}} = 0.04$). Opportunely, the protective T allele is common (MAF=0.49 in Europeans; 0.85 in African Americans; 0.61 in Latin Americans)¹¹⁶ and thus most of the population may benefit

from aspirin use. Taken together, these data support a mechanistic connection between aspirin chemoprevention and the WNT– β -catenin signalling axis, the functional relevance of 8q24 genetic variation in carcinogenesis and the potential utility of rs6983267 as a mechanistic biomarker.

Inflammatory and immune responses. The anti-inflammatory properties of aspirin and the establishment of chronic inflammation as a risk factor for CRC have elicited possible mechanistic connections between aspirin chemoprevention, chronic inflammation and modulation of the host immune response⁶³. PTGS2 can be induced at sites of inflammation in response to cytokines (such as interleukin-1 α (IL-1 α), IL-1 β , interferon- γ and tumour necrosis factor (TNF)) produced by inflammatory cells (FIG. 2). Additionally, PGE₂ seems to mediate inflammation-associated tumorigenesis in *Apc*^{Min/+} mouse models of CRC¹¹⁷. During inflammation-associated tumorigenesis, infiltrating neutrophils and tumour-associated fibroblasts were found to express EP2, TNF, IL-6, C-X-C motif chemokine 1 (CXCL1), PTGS2 and WNT5A¹¹⁸.

Multiple studies have examined the association of CRC risk and circulating inflammatory or immune markers, including IL-6, C-reactive protein (CRP) and soluble TNF receptor 2 (sTNFR2), in the context of aspirin use in human populations. A nested case–control study of women found that plasma sTNFR2 levels, but not IL-6 or CRP levels, were associated with risk of CRC (RR=1.67; 95% CI 1.05–2.68)¹¹⁹. Among those with high baseline levels of sTNFR2, the use of aspirin or other NSAIDs was associated with lower risk of CRC (RR=0.39; 95% CI 0.18–0.86). By contrast, women with low baseline levels of sTNFR2 did not

experience a preventive benefit associated with initiation of regular use of aspirin or NSAIDs (RR=0.86; 95% CI 0.41–1.79). These results, however, were not observed in a separate study of men¹²⁰. It remains unclear whether a true sex difference exists or whether this inconsistency stems from methodological differences between the studies. Furthermore, as shown in recent meta-analyses, the association of IL-6 and CRP levels with CRC has been inconsistent and no studies have reported a significant differential association of aspirin use with CRC according to levels of these markers^{121,122}.

The circulating inflammatory cytokine macrophage inhibitory cytokine 1 (MIC1; also known as growth differentiation factor 15 (GDF15)) may be an important mediator in systemic inflammatory responses and, as a member of the human transforming growth factor- β (TGF β) superfamily, may have a specific role in colorectal carcinogenesis^{123,124}. A recent cohort study examined plasma levels of MIC1 in the context of CRC risk¹²⁵. In a comparison of extreme quintiles, higher MIC1 levels were associated with a 93% increased risk of CRC (HR=1.93; 95% CI 1.27–2.94; $P_{\text{trend}} = 0.004$). Furthermore, in exploratory analyses, among individuals with high plasma MIC1 levels, the use of aspirin and NSAIDs was associated with a lower risk of PTGS2-positive (RR=0.60; 95% CI 0.41–0.88) but not PTGS2-negative CRC (RR=1.21; 95% CI 0.71–2.07).

GWASs have further implicated a potential role for aspirin in the modulation of immunity and inflammation. The SNP rs16973225 was identified in a genome-wide case-only G \times E interaction analysis¹². This SNP is located at chromosome 15q25.2, approximately 625 kb upstream of the gene encoding IL-16, which is a multifunctional cytokine with crucial functions in pro-inflammatory processes that has been implicated in inflammatory bowel disease and CRC. This SNP showed a genome-wide significant interaction with use of aspirin and/or NSAIDs ($P_{\text{interaction}} = 8.2 \times 10^{-9}$). Regular use was associated with a lower risk of CRC among individuals with the AA genotype (OR=0.66; 95% CI 0.62–0.71; $P = 1.9 \times 10^{-30}$). However, the reduced risk associated with regular aspirin use was nullified among those with the AC or CC genotype (OR=0.97; 95% CI 0.78–1.20). Taken together, these data suggest that the anticancer effect of aspirin may be mediated at least partially through modulation of chronic inflammation and the immune system.

Platelet-mediated effects. Unlike epithelial cells, which express both PTGS1 and PTGS2, platelets express only PTGS1 and, as non-nucleated cells, lack the ability to

resynthesize the enzyme¹¹. Thus, low-dose aspirin leads to permanent inhibition of platelet-mediated production of TXA₂ (FIG. 2). This action has been hypothesized

to explain the vascular benefits of aspirin, as TXA₂ is the major metabolite that promotes activation and aggregation of platelets, vasoconstriction and vascular smooth muscle

Table 3 | Summary of promising molecular biomarkers associated with aspirin chemoprevention of adenoma or colorectal cancer

Biomarker	Study population	Study design	Outcome	Cases (n)	Biomarker description	RR (95% CI)* of outcome according to regular aspirin or NSAID use stratified by biomarker		Refs
Germline genetic								
rs6983267-T	NHS and HPFS	Nested case-control	CRC	840	T allele of SNP	• TT: 0.52 (0.35–0.78) • GT: 0.61 (0.47–0.79)	GG: 0.99 (0.70–1.40)	115
rs2965667-TT	10 cohorts [‡]	GWAS [§]	CRC	8,634	TT genotype of SNP	TT: 0.63 (0.59–0.68)	TA or AA: 1.76 (1.16–2.66)	12
rs16973225-AA					AA genotype of SNP	AA: 0.63 (0.59–0.68)	AC or CC: 0.93 (0.75–1.17)	
rs2920421-GA	CCFR	Case-unaffected-sibling-control study	CRC	1,621	GA genotype of SNP	GA: 0.60 (0.45–0.80)	• GG: 1.12 (0.86–1.46) • AA: 0.83 (0.45–1.51)	94
rs2430420-GG	AFPPS	Nested cohort within an RCT	Adenoma	370	GG genotype of SNP	GG: 0.68 (0.50–0.94)	GA or AA: 0.95 (0.75–1.20)	154
rs28362380-TT					TT genotype of SNP	TT: 0.75 (0.61–0.92)	TC or CC: 1.32 (0.85–2.06)	
Colonic mucosa								
HPGD	NHS and HPFS	Prospective cohort	CRC	270	HPGD mRNA expression in normal mucosa	High HPGD: 0.49 (0.34–0.71)	Low HPGD: 0.90 (0.63–1.27)	65
Colorectal tumour								
PIK3CA mutation	NHS and HPFS	Prospective cohort	CRC	1,226	PIK3CA (exons 9 and 20) mt tumours	PIK3CA mt: 0.66 (0.48–0.89)	PIK3CA wt: 0.79 (0.69–0.90)	74,96
			CRC-specific survival	964		PIK3CA mt: 0.18 (0.06–0.61)	PIK3CA wt: 0.96 (0.69–1.32)	
BRAF mutation	NHS and HPFS	Prospective cohort	CRC	1,226	BRAF ^{V600E} mutation status of tumours	BRAF wt: 0.73 (0.64–0.83)	BRAF mt: 1.03 (0.76–1.38)	74
PTGS2 overexpression	NHS and HPFS	Prospective cohort	CRC	632	Positive PTGS2 expression in tumour by IHC	Positive PTGS2: 0.64 (0.52–0.78)	Negative PTGS2: 0.96 (0.73–1.26)	66
Urine								
PGE-M	NHS	Nested case-control	Adenoma	420	Quartiles (Q) of urinary PGE-M	High PGE-M (Q2–Q4): 0.61 (0.43–0.87)	Low PGE-M (Q1): 1.05 (0.50–2.19)	86
	AFPPS	RCT	Adenoma	328	Tertiles (T) of urinary PGE-M	High PGE-M (T2 or T3): • 81 mg: 0.85 (0.67–1.08) • 325 mg: 1.03 (0.82–1.29)	Low PGE-M (T1): • 81 mg: 0.62 (0.41–0.92) • 325 mg: 0.63 (0.43–0.91)	87
Plasma								
MIC1	NHS and HPFS	Nested case-control	CRC	618	Cohort-specific quintiles of plasma MIC1	High MIC1 and PTGS2 positive: 0.60 (0.41–0.88)	High MIC1 and PTGS2 negative: 1.21 (0.71–2.07)	175
sTNFR2	NHS	Nested case-control	CRC	280	≥Median plasma sTNFR2	sTNFR2 ≥ median: 0.39 (0.18–0.86)	sTNFR2 < median: 0.86 (0.41–1.79)	119

AFPPS, Aspirin/Folate Polyp Prevention Study; CI, confidence interval; CRC, colorectal cancer; GWAS, genome-wide association study; HPGD, 15-hydroxyprostaglandin dehydrogenase; HR, hazard ratio; IHC, immunohistochemistry; MIC1, macrophage inhibitory cytokine 1; mt, mutant; NSAID, non-steroidal anti-inflammatory drug; PGE-M, major metabolite of prostaglandin E₂; PIK3CA, PI3K catalytic subunit-α; PTGS2, prostaglandin-endoperoxide synthase 2; RCT, randomized clinical trial; RR, relative risk; SNP, single nucleotide polymorphism; sTNFR2, soluble tumour necrosis factor receptor 2; wt, wild-type. *All estimates are based on multivariable adjusted models. [‡]Ten cohorts: CCFR, Colon Cancer Family Registry; DACHS, Darmkrebs: Chancen der Verhütung durch Screening Study; DALIS, Diet, Activity and Lifestyle Study; HPFS, Health Professionals Follow-up Study; NHS, Nurses' Health Study; OFCCR, Ontario Familial Colorectal Cancer Registry; PMH-CCFR, Postmenopausal Hormone Study-CCFR; PLCO, Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial; VITAL, Vitamins and Lifestyle; WHI, Women's Health Initiative. [§]Case-only interaction analysis; ^{||}RR represents 81 mg aspirin versus placebo. No significant effect was measured for 325 mg aspirin.

cell proliferation¹²⁶. Recently, the anticancer effects of aspirin have been proposed to be mediated by platelets rather than through a direct effect on epithelial cells¹¹. Such a hypothesis would explain why aspirin, at

least in secondary analyses of RCTs of aspirin for CVD prevention, seems to be effective in preventing cancer even at low doses, which would be rapidly metabolized and would generate systemic concentrations not

expected to be high enough to directly inhibit epithelial PTGS2 and subsequent production of systemic PGE₂. To reconcile this with the highly consistent evidence implicating the importance of PTGS2 in colorectal carcinogenesis, it has been hypothesized that the recruitment of activated platelets to the mucosa in response to inflammatory events or mucosal injury leads to paracrine upregulation of PTGS2 expression in epithelial cells^{11,127}. Thus, even low doses of aspirin, typically considered only sufficient to inhibit platelets, may eventually lead to substantial downstream inhibition of PTGS2 in epithelial and tumour cells.

Additional mechanisms: insights from survival studies. Beyond its effect on cancer initiation, aspirin may also have an effect on cancer progression. Several studies have shown that aspirin use, particularly after diagnosis, is associated with a lower risk of CRC-specific mortality among patients with established disease^{10,14,128–133}. These associations may also be explained by the effect of aspirin on pathways such as PG synthesis or WNT signalling. For example, aspirin was associated with improved survival, particularly among individuals with index primary tumours that overexpressed PTGS2 by immunohistochemistry compared with those with tumours without PTGS2 overexpression¹²⁸. However, there may be additional distinct pathways by which aspirin influences cancer progression or metastasis but not initiation^{134,135}. For example, aspirin use does not seem to be differentially associated with the likelihood of developing cancer with activating mutations in *PIK3CA* (which encodes PI3K catalytic subunit- α)⁷⁴, the product of which functions in a pathway that seems to share functionally important crosstalk with PTGS2 (REF. 136). However, after diagnosis, aspirin use was associated with dramatically improved survival among individuals with *PIK3CA*-mutant CRC in a cohort study (HR = 0.18; 95% CI 0.06–0.61)⁹⁶ and in an analysis of self-selected aspirin use within a clinical trial of adjuvant rofecoxib (HR = 0.11; 95% CI 0.001–0.83)¹³⁷, supporting the possibility that aspirin may influence distinct biological pathways in the setting of established cancer. Two additional observational studies did not individually support these findings^{138,139}. However, a recent meta-analysis¹⁴⁰, which included one of these null studies¹³⁸, concluded that the association of aspirin with CRC-specific survival does seem to differ according to *PIK3CA* mutation status. Additional RCTs

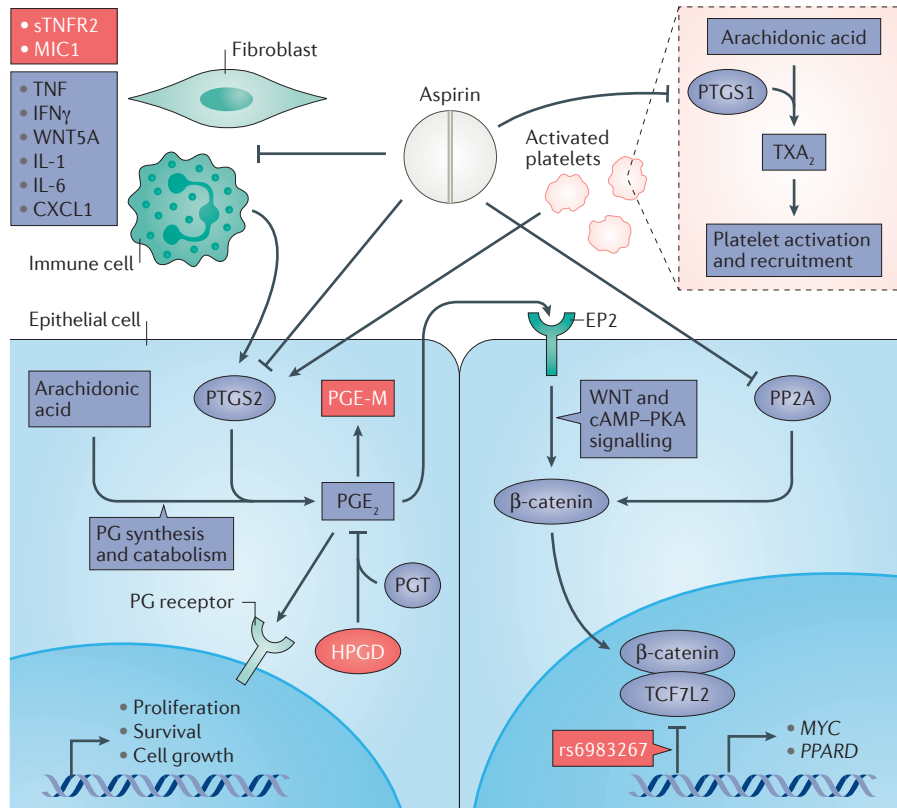


Figure 2 | The hypothesized inter-related mechanisms of aspirin chemoprevention. Aspirin exerts its anticancer effects through several interconnected mechanisms: prostaglandin (PG) synthesis and catabolism in epithelial cells (lower left panel); inhibition of WNT- β -catenin signalling (lower right panel); and inactivation of platelets (upper right panel) and the host immune response (upper left panel). Through direct inhibition of prostaglandin-endoperoxide synthase 2 (PTGS2) at higher doses, aspirin blocks conversion of arachidonic acid to PGE₂. Aspirin's effects may be enhanced by the expression of 15-hydroxyprostaglandin dehydrogenase (HPGD), a metabolic antagonist of PTGS2, through catabolism of PGE₂ to PGE-M (the major metabolite of PGE₂). PGE₂ can activate WNT- β -catenin signalling through paracrine activation of EP2, its plasma membrane receptor. PGE₂ can also activate cAMP and protein kinase A (PKA) signalling, further stabilizing cytosolic β -catenin. Aspirin can further inhibit β -catenin through inactivation of protein phosphatase 2A (PP2A), the phosphatase responsible for removing post-translational modifications that target β -catenin for ubiquitylation and subsequent destruction. In colorectal tumorigenesis, PTGS2 and β -catenin are upregulated, leading to increased cellular proliferation, growth and survival. Once in the nucleus, β -catenin forms a transcriptional activation complex with transcription factor 7 like-2 (TCF7L2) and activates effector genes with roles in tumorigenesis, such as *MYC* and *PPARD* (which encodes peroxisome proliferator-activated receptor- δ). Genetic variation at the single nucleotide polymorphism (SNP) rs6983267 may impair binding of the β -catenin-TCF7L2 complex to these transcriptional targets. The antiplatelet effects associated with low-dose aspirin are mediated through inhibition of PTGS1. PTGS1 converts arachidonic acid to thromboxane A₂ (TXA₂), the major metabolite promoting recruitment and activation of platelets. Platelets and inflammatory cells — especially infiltrating neutrophils and fibroblasts — recruited to the colonic epithelium in response to chronic inflammation or mucosal injury, may act as paracrine activators of PTGS2 in the colonic epithelium. The combined antiplatelet and anti-inflammatory effects of aspirin may specifically prevent inflammation-associated tumorigenesis, and aspirin may be particularly effective in individuals with increased circulating levels of inflammatory cytokines. Targets that are discussed as potential biomarkers that may have utility in risk stratification for precision chemoprevention are shown in red. CXCL1, C-X-C motif chemokine 1; IFN γ , interferon- γ ; IL, interleukin; MIC1, macrophage inhibitory cytokine 1; PGT, prostaglandin transporter; sTNFR2, soluble TNF receptor 2; TNF, tumour necrosis factor.

(ASCOLT³⁴ and ADD-ASPIRIN¹⁴¹) designed to assess the effect of aspirin in patients who have undergone potentially curative surgery for CRC are currently underway and will provide additional opportunities to determine whether *PIK3CA* or other molecular biomarkers of cancer-specific pathways may be leveraged to predict responsiveness to therapy.

Additional markers for risk stratification.

The results of three RCTs of aspirin in relation to CRC biomarkers have elucidated other potential markers for molecular risk stratification that are associated with alternative pathways. First, a Phase IIb double-blind, placebo-controlled trial among patients with a history of adenoma or CRC examined the effect of aspirin at 325 mg per day for 3 months on spectral biomarkers in macroscopically normal tissue that measure cellular microarchitectural changes associated with the development of neoplasia^{53,142,143} (TABLE 4). Following aspirin treatment, both spectral slope (SPEC) and fractal dimension (FRAC) trended towards less neoplastic signatures in normal tissue, although only SPEC approached statistical significance⁵³. In secondary analyses, tissue expression of PGE₂ correlated with SPEC and FRAC in aspirin users, but there was no differential association according to *UGT1A6* (which encodes UDP glucuronosyltransferase 1 family polypeptide A6) genotype.

Second, the Aspirin and the Biology of the Colon (ABC) placebo-controlled crossover trial examined the impact of

aspirin treatment for 60 days on colonic epithelial and stromal gene expression among healthy participants predicted to have impaired aspirin glucuronidation on the basis of *UGT1A6* genotype⁵⁴ (TABLE 4). Overall, aspirin did not induce differences in gene expression but did decrease tissue expression of PGE₂. A recent secondary metabolomic analysis of plasma collected from these participants identified a potentially interesting association between aspirin use and a reduction in the levels of the oncometabolite 2-hydroxyglutarate¹⁴⁴.

Last, aspirin has been hypothesized to act synergistically with ornithine decarboxylase (ODC) inhibitors¹⁴⁵. ODC is the enzyme responsible for the synthesis of colonic polyamines, which have been consistently associated with the development of colorectal neoplasia^{146,147}. An RCT to determine the effect of combined treatment with the ODC inhibitor eflornithine and aspirin on adenoma recurrence is currently underway¹⁴⁸ and may build on the success of the earlier eflornithine–sulindac combination trial in preventing adenoma recurrence¹⁴⁹. Previously, the A allele of SNP rs2302615 in the *ODC* promoter has been associated with adenoma risk^{150–152}, but its utility in predicting aspirin sensitivity has been inconsistent^{110,153,154}. Most recently, among a sub-cohort of the AFPPS RCT, a targeted genotyping approach that examined SNPs in the *ODC* promoter identified two novel SNPs, the GG genotype of rs2430420 and the TT genotype of rs28362380, that seem to be potential markers of efficacy with 81 mg per day of aspirin¹⁵⁴ (TABLE 3).

However, this genetic interaction was not observed with the group receiving a dose of 325 mg. Clearly, studies in additional large populations are warranted to validate the use of *ODC* SNPs, SPEC and FRAC, and 2-hydroxyglutarate as potential risk stratification biomarkers.

Conclusions

The evidence in support of the effect of aspirin on CRC is compelling, forming the basis for a recent clinical recommendation for its use in chemoprevention. We, and others, have shown that the effect of aspirin on CRC is not likely to be mediated by a single mechanism, but instead by several inter-related mechanisms (FIG. 2). Specifically, aspirin may inhibit PTGS-mediated conversion of arachidonic acid to PGE₂ either by a direct effect on epithelial cells or through a paracrine effect via inhibition of PTGS1-mediated synthesis of TXA₂ in platelets. Inhibition of PGE₂ synthesis by aspirin may be enhanced by background upregulation of *HPGD*. Aspirin may also inhibit WNT signalling either directly or through downregulation of PGE₂.

Several genetic, tissue, plasma and urinary biomarkers relevant to these multiple, yet inter-related, pathways seem to have promise as biomarkers for risk stratification to identify those individuals who are most likely to benefit from a long-term aspirin regimen. Nonetheless, despite their potential, several pragmatic test characteristics — including specificity, sensitivity and convenience — are crucial considerations for their adoption in

Table 4 | Placebo-controlled clinical trials of aspirin with biomarker end points

Trial name	Primary end point	Inclusion criteria	Aspirin dose (mg per day)	Participants (n)	Duration of treatment	Primary end point	Secondary end points
ABC	Tissue gene expression	Healthy men and women aged 20–45 years who completed a cross-sectional study of diet and aspirin metabolism and were selected on the basis of <i>UGT1A6</i> genotype	325	44	60 days	Null; no significant differences in gene expression were observed by aspirin use or <i>UGT1A6</i> genotype ⁵⁴	PGE ₂ tissue expression was lower in individuals receiving aspirin than in those receiving placebo ($P < 0.001$) ⁵⁴ . Plasma concentrations of 2-hydroxyglutarate was reduced after aspirin use ($P = 0.005$) ¹⁴⁴
Spectral Markers in Aspirin Chemoprevention of Colonic Neoplasia ⁵³	SPEC and FRAC	Subjects aged ≤70 years undergoing surveillance colonoscopy for adenoma or CRC resected in the past 6 years	325	72	3 months	SPEC = +48.9% from baseline (less neoplastic), $P = 0.055$; FRAC = –48.9% from baseline (less neoplastic), $P = 0.200$	PGE ₂ suppression correlated with SPEC ($R = -0.55$; $P = 0.01$), but not FRAC; no significant differences in SPEC or FRAC according to <i>UGT1A6</i> genotype

ABC, Aspirin and the Biology of the Colon; CRC, colorectal cancer; FRAC, fractal dimension; PGE₂, prostaglandin E₂; R, Spearman's correlation coefficient; SPEC, spectral slope; UGT, UDP-glucuronosyltransferase.

clinical settings. For example, urine- and blood-based biomarkers may be relatively easy to use in the clinic. However, the markers identified to date (such as MIC1 or PGE-M) may lack specificity for neoplasia as they may be generally elevated in response to other inflammatory states. Tissue biomarkers (such as HPGD) may offer greater specificity than urine- or blood-based biochemical markers, but they require invasive biopsy and may be more challenging to obtain in primary prevention settings. Finally, the sensitivity and specificity of germline genetic markers are likely to depend not only on the strength of interaction with aspirin use but also on the allele frequency of a polymorphism in a given population. Moreover, genetic testing may be less easily implemented in clinical settings, given the additional need for interpretation of genetic information and patient counselling¹⁵⁵. Thus, further studies are required to prioritize the biomarkers identified to date for implementation in the clinic and to identify additional novel pathways and biomarkers.

To help achieve this goal, we recently launched the ASPIRED trial³⁶. Participants who have recently had an adenoma removed will be enrolled in a double-blind, placebo-controlled RCT of 81 mg or 325 mg of aspirin daily for 8 weeks. Individuals will provide blood, urine, colorectal biopsy, saliva and stool specimens both before and after treatment. These biological specimens will then be used to examine the effect of aspirin on cancer-related biomarkers, including many of those discussed in this Opinion article. Additional ongoing RCTs, including the seAFOod polyp prevention trial³⁸, will also examine the effect of aspirin and fish oil on bioactive lipid mediators, including urinary levels of PGE-M¹⁵⁶. Finally, RCTs involving patients at risk for cardiovascular events (ARRIVE³³) or other primary outcomes (ASPREE³⁵) may also provide additional evidence to support the efficacy of aspirin in cancer prevention.

In conclusion, the recent USPSTF draft recommendation that recognizes the weight of evidence in support of aspirin for CRC prevention is a crucial first step in realizing a potential broader population-wide impact of aspirin use for chemoprevention. However, it is likely that the future of aspirin chemoprevention will still benefit from molecularly inspired human research aimed at clarification of aspirin's inter-related anticancer mechanisms. Such studies will be crucial for fulfilling the promise of precision medicine for cancer prevention.

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Competing interests statement

The authors declare [competing interests](#): see Web version for details.

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American College of Cardiology and American Heart Association risk calculator: <http://www.cvriskcalculator.com>
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