Cyclooxygenase Inhibitors and Cancer: The Missing Pieces

Carlo Patrono

Department of Pharmacology, Catholic University School of Medicine, Rome, Italy

Corresponding Author: Carlo Patrono, MD
Istituto di Farmacologia
Università Cattolica del S. Cuore
Largo Francesco Vito, 1
00168 Rome
Italy
Email address for correspondence: carlo.patrono@unicatt.it
Running Title: Cyclooxygenase Inhibitors and Cancer

Number of text pages, 33
5 Figures
52 References
Abstract: 193 words
Introduction: 218 words
Sections 2 to 9: 4717 words

List of nonstandard abbreviations used in the paper, with abbreviations listed in alphabetical order:
CI, confidence interval
COX, cyclooxygenase
DSMB, data and safety monitoring board
HR, hazard ratio
IPD, individual participant data
NSAIDs, non-steroidal anti-inflammatory drugs
OR, odds Ratio
Prostaglandin, PG
RCT, randomized controlled trial
RR, relative risk
Thromboxane, TX

Recommended section assignment: Other
Abstract

At 125, aspirin still represents the cornerstone of anti-platelet therapy for the acute treatment and long-term prevention of atherothrombosis. The development of a selective regimen of low-dose aspirin for the inhibition of platelet thromboxane production was key to maximizing its antithrombotic efficacy and minimizing its gastrointestinal toxicity. Based on about 50 observational studies, published over the past 30 years, aspirin and other cyclooxygenase inhibitors have been associated with a reduced risk of colorectal cancer, and possibly other digestive tract cancers. The apparent chemopreventive effect of aspirin has been confirmed in post-hoc analyses of randomized cardiovascular trials and their meta-analyses. Moreover, prevention of sporadic colorectal adenoma recurrence was demonstrated by randomized controlled trials of low-dose aspirin and selective cyclooxygenase-2 inhibitors. A single placebo-controlled randomized trial of aspirin has shown long-term colorectal cancer prevention in patients with the Lynch syndrome. The sequential involvement of thromboxane-dependent platelet activation and cyclooxygenase-2-driven inflammatory response in the early stages of colorectal carcinogenesis may explain these clinical benefits. The aim of this mini-review is to analyze the existing evidence for a chemopreventive effect of aspirin and other cyclooxygenase inhibitors and discuss the missing pieces of this mechanistic and clinical puzzle.

Significance Statement

Low-dose aspirin and other cyclooxygenase inhibitors have been associated with a reduced risk of colorectal cancer, and possibly other digestive tract cancers. The sequential involvement of
thromboxane-dependent platelet activation and cyclooxygenase-2-driven inflammatory response in the early stages of colorectal carcinogenesis may explain these clinical benefits. The aim of this mini-review is to analyze the evidence for a chemopreventive effect of aspirin and other cyclooxygenase inhibitors and discuss the missing pieces of this mechanistic and clinical puzzle.

1. Introduction

The year 2022 marked the 125th anniversary of the first synthesis of acetylsalicylic acid within an industrial environment (Patrono, 2023). Despite its age, aspirin still represents the cornerstone of anti-platelet therapy for the treatment and prevention of atherothrombosis (Patrono et al., 2017). The development of a selective regimen of low-dose aspirin for the inhibition of platelet thromboxane production was key to maximizing its antithrombotic efficacy and minimizing its gastrointestinal toxicity (Patrono et al., 2005).

Precision antiplatelet therapy with optimized aspirin dosing regimens is currently being developed in relatively rare diseases characterized by abnormal platelet turnover, such as essential thrombocythemia (Rocca et al., 2020).

Moreover, measurement of platelet activation through non-invasive biomarkers and use of low-dose aspirin as an investigative tool allowed broadening our understanding about the protean roles of platelets in health and disease, including cancer (Davì and Patrono, 2007; Patrono, 2015; Patrignani and Patrono, 2016). The sequential involvement of thromboxane (TX)-dependent platelet activation and cyclooxygenase (COX)-2-driven inflammatory response in the early stages of colorectal carcinogenesis may explain the remarkably similar chemopreventive properties of low-dose aspirin and COX-2 inhibitors (Patrono et al, 2001; Patrignani and Patrono, 2016).
The aim of this mini-review is to analyze the existing evidence for a chemopreventive effect of aspirin and other COX-inhibitors and discuss the missing pieces of this mechanistic and clinical puzzle.

2. Observational studies

Based on over 50 observational studies, published over the past 30 years, aspirin has been associated with a reduced risk of colorectal cancer, and possibly other digestive tract cancers (Bosetti et al., 2020). To provide a quantification of this association and characterize its determinants, a systematic review and meta-analysis of observational studies on aspirin and cancers of the digestive tract was performed by Bosetti et al. (2020). They estimated the pooled relative risk (RR) of cancer for regular aspirin use versus non-use with random-effects models, and investigated the dose- and duration-risk relations.

Regular aspirin use was associated with 20% to 40% reduced risk of the following cancers: colorectal (RR, 0.73; 95% confidence interval [CI], 0.69-0.78; 45 studies), squamous-cell esophageal (RR, 0.67; 95% CI, 0.57-0.79; 13 studies), adenocarcinoma of the esophagus and gastric cardia (RR, 0.61; 95% CI, 0.49-0.77; 10 studies), stomach (RR, 0.64; 95% CI, 0.51-0.82; 14 studies), hepato-biliary tract (RR, 0.62; 95% CI, 0.44-0.86; 5 studies), and pancreatic cancer (RR, 0.78; 95% CI, 0.68-0.89; 15 studies) (Bosetti et al., 2020). The associations were stronger in case-control than in cohort and nested case-control studies. Risk estimates were consistent across various selected covariates. For all neoplasms, inverse duration-risk relations with aspirin use were found (Bosetti et al., 2020).

The evidence for a similar protective association of regular aspirin use with other cancers is much less convincing. To provide a quantification of the potential effects of aspirin on seven non-
gastrointestinal cancers, Santucci et al. (2021) performed a systematic review and meta-analysis of observational studies, with a similar design as the study of digestive tract cancers (Bosetti et al., 2020). Regular aspirin use was associated with 7% to 12% reduced risk of lung, breast, endometrial, ovarian and prostate cancer (Santucci et al., 2021) (Figure 1). However, for most cancer locations, nonsignificant associations were reported in cohort and nested case-control studies, and there was substantial between-study heterogeneity. No association was reported for bladder and kidney cancer. No duration-risk relations were observed for most neoplasms, except for an inverse duration-risk relation for prostate cancer (Santucci et al, 2021).

In a nationwide population study of patients with chronic viral hepatitis in Sweden, use of low-dose aspirin (75 mg or 160 mg daily) was associated with a substantially lower risk of incident hepatocellular carcinoma and lower liver-related mortality than no use of aspirin (Simon et al, 2020). The apparent benefits of aspirin were duration-dependent, with one-third lower risk of hepatocellular carcinoma after 3 to 5 years of use than with short-term use. These benefits were not accompanied by a significantly higher incidence of gastrointestinal bleeding (Simon et al, 2020). Aspirin may act as an adjuvant to other therapies (e.g., in combination with sorafenib) in reducing hepatocellular carcinoma recurrence (Ricciotti et al, 2021).

Several aspects of the observational evidence summarized above should be considered. The first is represented by the remarkable consistency of a large number of epidemiologic studies in reporting about a one-quarter reduction in risk of colorectal cancer associated with regular aspirin use (Bosetti et al., 2020), and similar effects on other digestive tract cancers (20% to 40% risk reduction, depending on specific location) (Bosetti et al., 2020). Secondly, a similar apparent protection had been previously associated with sustained use of other traditional non-steroidal anti-inflammatory drugs (NSAIDs), based on numerous epidemiologic studies in the general population (Thun et al., 2002). The results of the latter studies suggest a mechanism-based class
effect of COX inhibitors, and indicate that the duration and continuity of NSAID use may be more critical than the daily dose (Thun et al., 2002). Thirdly, given the possibility that the chemopreventive effect of aspirin may be mediated, at least in part, through its antiplatelet effect (Patrano et al., 2001; see below), one should look for a potential association with other antiplatelet agents. In a nested case-control study of a primary care database in Spain, Rodríguez-Miguel et al. (2019) found the use of clopidogrel, a platelet P2Y12 blocker, to be associated with 20% to 30% reduced risk of colorectal cancer, an effect similar to that of low-dose aspirin. More studies are clearly needed to confirm or challenge this interesting observation.

Although one should acknowledge the limitations of observational studies in establishing a causal relationship between drug use and outcomes, it should also be noted that observational studies have yielded largely similar estimates of gastrointestinal and cardiovascular risks associated with NSAIDs as established by a large meta-analysis of all NSAID RCTs (CNT Collaboration, 2013).

3. **Post-hoc analyses of randomized cardiovascular trials**

In light of the above observational evidence, Flossmann and Rothwell (2007) examined the effects of aspirin on risk of colorectal cancer in two cardiovascular RCTs with available post-trial follow-up for more than 20 years: the British Doctors Aspirin Trial (BDAT, n=5139, two-thirds assigned 500 mg aspirin for 5 years, a third open control), a primary prevention study in apparently healthy men, and the UK-TIA Aspirin Trial (n=2449, two-thirds allocated 300 mg or 1200 mg aspirin for 1–7 years, a third placebo control), a secondary prevention study in patients with cerebrovascular disease. Colorectal cancer incidence was not a pre-specified secondary
endpoint of these trials, hence the results of these post-hoc analyses should be interpreted with caution.

In these RCTs, earlier randomization to aspirin appeared to reduce the long-term incidence of colorectal cancer (pooled hazard ratio [HR], 0.74; 95% CI, 0.56-0.97; P=0.02 overall; 0.63, 0.47-0.85; P=0.002 if assigned aspirin for 5 years or longer). However, this effect was only apparent after a latency of 10 years and was dependent on duration of scheduled trial treatment and compliance (Figure 2). No significant effect on incidence of non-colorectal cancers was observed (Flossmann and Rothwell, 2007).

Similar post-hoc analyses of long-term colorectal cancer incidence and mortality were extended to two additional cardiovascular RCTs of aspirin 75 mg daily, the Thrombosis Prevention Trial (TPT, n=5085), a primary prevention study in high-risk men, and the Swedish Aspirin Low Dose Trial (SALT, n=1360), a secondary prevention study in patients with cerebrovascular disease (Rothwell et al., 2010) (Figure 2). During a median follow-up of 18.3 years, allocation to aspirin in the four RCTs (BDAT, UK-TIA, TPT and SALT) reduced the 20-year risk of colon cancer (incidence HR, 0.76; 95% CI, 0.60-0.96; P=0.02; mortality HR, 0.65; 95% CI, 0.48-0.88; P=0.005), but not rectal cancer. There was no apparent increase in protection at doses of aspirin higher than 75 mg daily (Rothwell et al., 2010). From a mechanistic point of view, it is interesting to note that TPT used a low-dose, controlled-release aspirin formulation with substantially reduced systemic bioavailability, developed to achieve cumulative inhibition of platelet COX-1 in the pre-hepatic circulation while sparing COX-2 in the systemic vascular endothelium (Clarke et al., 1991); and yet the estimated effect of aspirin on long-term risk of death due to colorectal cancer in TPT was at least as large as in the 1,200 mg arm of the UK-TIA trial (Rothwell et al., 2010).

In further post-hoc analyses, Rothwell et al. (2012) obtained individual participant data (IPD) from six RCTs of daily aspirin versus no aspirin in primary prevention of vascular events. In the six
trials involving 35,535 participants, aspirin reduced cancer incidence from 3 years onwards (Odds Ratio [OR], 0.76; 95% CI, 0.66-0.88; P=0.0003), suggesting earlier chemopreventive effects than previously reported.

Based on IPD meta-analyses of ten RCTs of aspirin in primary prevention (including 117,279 participants), Rothwell et al. (2018) reported that aspirin-associated reductions in long-term risk of colorectal cancer were influenced by body weight (P for interaction=0.038). However, based on observational analysis of UK primary care data, Cea Soriano et al. (2019) reported that the effects of low-dose aspirin in reducing colorectal cancer risk were not modified by body weight or body mass index.

No comparable analyses of long-term colorectal cancer incidence and mortality have been performed in the follow-up of participants in P2Y12-blocker trials, most likely reflecting the relatively short duration of such trials as well as the nature of the randomized comparison, mostly versus aspirin or placebo on top of aspirin (Patrono et al, 2017).

4. Prospective, long-term analyses of cancer incidence and mortality as secondary endpoints of cardiovascular trials

A primary prevention RCT of aspirin (100 mg every other day) for cardiovascular prevention in apparently healthy women, the Women’s Health Study (WHS, n=39,876), prespecified colorectal cancer as a secondary endpoint, and assessed its incidence beyond the 10-year scheduled duration of randomized treatment by including post-trial observational follow-up (Cook et al., 2013). Although the WHS reported a borderline significantly lower incidence of colorectal cancer at 17.5 years of follow-up (OR, 0.82; 95% CI, 0.69-0.98) (Figure 2), more recent unpublished data (cited by the US Preventive Services Task Force, 2022) showed that this effect did not persist from
17.5 to 26 years of follow-up. As noted by AT Chan (2022), it is plausible that the benefit of aspirin in preventing colorectal cancer might be attenuated with longer observational follow-up, due to cohort attrition and the potential influence of time-dependent, post-trial self-selected aspirin use.

Three more recent, low-dose (100 mg daily) aspirin RCTs for primary cardiovascular prevention or to improve disability-free survival, not included in the meta-analyses of Rothwell et al (2012; 2018), prespecified cancer incidence and mortality as secondary endpoints. The Aspirin to Reduce Risk of Initial Vascular Events (ARRIVE, n=12,546) recruited patients categorized as being at moderate cardiovascular risk and followed them for about 5 years (Gaziano et al., 2018). The effects of aspirin on cancer incidence in this setting have not been reported yet.

A Study of Cardiovascular Events in Diabetes (ASCEND, n=15,480) enrolled adults who had diabetes mellitus but no evident cardiovascular disease and followed them for 7.4 years (The ASCEND Study Collaborative Group, 2018). During the scheduled duration of follow-up, there was no significant difference between subjects assigned low-dose aspirin and those assigned placebo in the incidence of gastrointestinal tract cancer (157 [2.0%] and 158 [2.0%] participants, respectively) or all cancers (897 [11.6%] and 887 [11.5%]). The two groups also did not differ significantly with regard to the risk of fatal or nonfatal cancer overall or at particular sites; longer-term follow-up for these outcomes is ongoing (The ASCEND Study Collaborative Group, 2018).

The Aspirin in Reducing Events in the Elderly (ASPREE, n=19,114) trial recruited community-dwelling persons in Australia and the United States who were 70 years of age or older (or ≥65 years of age among blacks and Hispanics in the United States) and did not have cardiovascular disease, dementia, or physical disability, and followed them for 4.7 years when the trial was stopped for futility (McNeil et al., 2018a). The risk of the secondary endpoint of death from any cause was higher in the aspirin group than in the placebo group (12.7 events and 11.1 events per 1000 person-years, respectively; HR, 1.14; 95% CI, 1.01 to 1.29), but not to an extent that reached
statistical significance if the P value was corrected for multiple comparisons (McNeil et al., 2018b). Cancer was the major contributor to the higher mortality in the aspirin group, accounting for 1.6 excess deaths per 1000 person-years (McNeil et al., 2018b).

However, when the ASPREE Investigator Group subsequently examined incident cancer events rather than cancer deaths, low-dose aspirin was not found to increase the incidence of overall cancer or colorectal cancer (McNeil et al., 2021). As noted by AT Chan (2022), an increase in cancer death after <5 years of exposure, in the absence of anatomic specificity or a corresponding increase in cancer incidence, is unexpected and diminishes the likelihood that the apparent association of aspirin with cancer mortality in ASPREE was causal. Also, as acknowledged by the ASPREE Investigator Group, “other primary prevention trials of aspirin have not identified similar results, which suggests that the mortality results reported here should be interpreted with caution” (McNeil et al., 2018b).

In fact, in an accompanying editorial, PM Ridker (2018) performed a tabular data meta-analysis of the effects of aspirin on all-cause mortality in 14 primary prevention RCTs (including ASPREE) and reported an overall HR of 0.97 (95% CI, 0.93-1.01), with the ASPREE point estimate of 1.14 clearly appearing as an outlier vis-à-vis the other 13 RCTs. Among these, the ASCEND trial recorded about 50% more deaths over a 50% longer follow-up than in ASPREE, and therefore had greater statistical power than ASPREE to detect a small excess in all-cause mortality due to aspirin. Although in a somewhat younger population than in ASPREE (63 vs 74 years) ASCEND showed an HR of 0.94 (95% CI, 0.85-1.04) for the effect of low-dose aspirin on all-cause mortality (Ridker, 2018).

Whether age is an important determinant of the effects of aspirin on all-cause mortality, and cancer mortality in particular, is currently being examined by IPD meta-analyses of the 14 primary
prevention RCTs, carried out by the Antithrombotic Trialists’ (ATT) Collaboration and Non-Vascular outcomes on Aspirin (NoVA) Collaboration, respectively.

5. Sporadic colorectal adenoma prevention trials

Four placebo-controlled RCTs of aspirin (81 to 325 mg daily) have shown a reduced risk of colorectal adenoma recurrence among approximately 3,000 individuals with a history of adenoma or colorectal cancer. A meta-analysis of these trials suggests a one-fifth reduction in any adenoma and a one-fourth reduction in advanced lesions, with no apparent dose-dependence of the chemopreventive effect during a median follow-up of about three years (Cole et al., 2009). The pooled risk ratio for any dose of aspirin vs placebo was 0.83 (95% CI, 0.72 to 0.96) for the endpoint of any adenoma, and 0.72 (95% CI, 0.57 to 0.90) for any advanced lesion. These trials provide evidence of causality for the reported associations of regular aspirin use and reduced risk of colorectal cancer, as adenomas are the precursors to the vast majority of cancers (Chan, 2022). Moreover, these findings suggest that aspirin may act at an early step in colorectal carcinogenesis, i.e., the progression from a presumably normal colorectal mucosa to an adenomatous lesion (Cole et al., 2009).

Two placebo-controlled RCTs, with a similar design as the four aspirin trials, evaluated the chemopreventive effects of the selective COX-2 inhibitors, rofecoxib and celecoxib (Baron et al., 2006; Bertagnolli et al., 2006). The Adenomatous Polyp PRevention On Vioxx (APPROVe) trial randomized 2,587 higher-risk subjects with a recent history of histologically confirmed adenomas to receive daily rofecoxib 25 mg or placebo (Baron et al., 2006). The primary endpoint was all adenomas diagnosed during a 3-year treatment. Adenoma recurrence was less frequent in subjects assigned to rofecoxib than in those randomized to placebo (RR, 0.76; 95% CI, 0.69-0.83;
P<0.0001). Rofecoxib similarly reduced the risk of advanced adenomas. However, during the treatment period, rofecoxib also increased the risk of serious cardiovascular events (RR, 1.89; 95% CI, 1.18-3.04), which led the Data and Safety Monitoring Board (DSMB) to recommend early termination of the APPROVe trial (Baron et al, 2006).

The Adenoma Prevention with Celecoxib (APC) trial randomly assigned 2,035 patients who had adenomas removed before study entry to receive a 3-year treatment with 200 mg or 400 mg of celecoxib or placebo twice daily (Bertagnolli et al., 2006). Because of a 2- to 3-fold increased incidence of cardiovascular events in the two celecoxib groups, use of the study medication was discontinued early, in compliance with the recommendations of the DSMB, at a time when 1762 patients (87%) had completed three years of treatment. The estimated cumulative incidence of one or more adenomas by year 3 was reduced by 33% to 45% by the two celecoxib regimens, with similar reductions in advanced adenomas, with no apparent increase in gastrointestinal ulceration and hemorrhage (Bertagnolli et al., 2006).

For both aspirin and coxibs, the largest risk reduction in adenoma recurrence appeared during the first year of treatment, with somewhat attenuated benefits thereafter. Mechanistically, it is interesting to note that the chemopreventive effect size of low-dose (81 to 160 mg daily) aspirin, largely sparing COX-2 activity, was comparable to that of rofecoxib 25 mg, largely sparing COX-1 activity (FitzGerald and Patrono, 2001). This finding may suggest either that the two COX-isozymes catalyze the formation of distinct and equally important prostanoids (e.g., TXA2 and PGE2) in the early stage(s) of colorectal carcinogenesis, or that activation of platelet COX-1 is responsible for COX-2 induction in adjacent nucleated cells of the intestinal mucosa (Patrono et al., 2001) (see below). Within the limitations of sub-group analysis of RCTs, the similar efficacy of rofecoxib and celecoxib in reducing adenomas in patients taking aspirin as in those not taking
aspirin (Baron et al., 2006; Bertagnolli et al, 2006) is consistent with additive effects resulting from simultaneous suppression of COX-1 and COX-2 activities.

6. Colorectal cancer prevention in patients with Lynch syndrome

The Cancer Prevention Programme (CaPP) was initiated in 1993 to investigate potential chemopreventive strategies in patients with genetic predisposition to colorectal and other cancers. The CAPP2 trial recruited approximately 700 subjects with the Lynch syndrome (also known as hereditary non-polyposis colon cancer), displaying a significant mutation in a DNA mismatch repair gene, who were assigned to receive enteric-coated aspirin 600 mg or placebo (and a resistant starch, or placebo corn starch) daily (Burn et al., 2008). Adenoma or carcinoma developed in 66 participants receiving aspirin (18.9%), as compared with 65 receiving placebo (19.0%) (RR, 1.0; 95% CI, 0.7 to 1.4), over a mean treatment period of 2.5 years (Burn et al., 2008).

The CAPP2 protocol had anticipated a delayed effect of aspirin on cancer incidence and provided for follow-up to 10 years. When the first participants reached their 10-year follow-up (mean follow-up, 4.6 years), intention-to-treat analysis showed a trend for reduced colorectal cancer incidence in the aspirin arm compared with placebo (HR, 0.63; 95% CI, 0.35-1.13; P=0.12). Per-protocol analysis, limited to participants completing at least 2 years of aspirin therapy, showed a statistically significant reduction in colorectal cancer (HR, 0.41; 95% CI, 0.19-0.86; P=0.02) and a similar effect on all Lynch syndrome cancers (Burn et al., 2011).

Furthermore, the cancer histories of all CAPP2 participants were reviewed where possible up to the planned 10-year follow-up, and a subset of English, Finnish, and Welsh participants were monitored via national registries for up to 20 years. Intention-to-treat Cox proportional hazards
analysis showed that earlier aspirin treatment protected against the primary endpoint of colorectal cancer (HR, 0.65; 95% CI, 0.43-0.97; P=0.035) (Burn et al., 2020) (Figure 2).

When examining the Kaplan-Meier curves for time to first colorectal cancer in the aspirin and placebo arms of CAPP2, the protective effect of aspirin does not become apparent for at least 6 to 8 years, and this delay is quite similar to the lag-time in the post-hoc analyses of aspirin cardiovascular RCTs (Flossmann and Rothwell, 2007; Rothwell et al., 2010), and in the long-term, post-trial follow-up of the WHS (Cook et al., 2013) (Figure 2).

Based on a review of the CAPP2 findings, the UK National Institute for Health and Care Excellence (NICE), issued a new recommendation under the heading 1.1 Reduction in risk of colorectal cancer in people with Lynch syndrome:

1.1.1 Consider daily aspirin, to be taken for more than 2 years, to reduce the risk of colorectal cancer in people with Lynch syndrome (NICE guideline [NG151] published 29 January 2020 at nice.org.uk; last updated: 15 December 2021).

7. A biologically plausible mechanism of action

Traditionally, inhibition of the inflammatory response has been considered the most likely mechanism of action of COX inhibitors underlying their chemopreventive effect against colorectal (and other) cancer (Thun et al., 2002; Thun et al., 2012). This hypothesis was supported by several lines of evidence: i) the role of inflammation in cancer (Bottazzi et al., 2018), possibly mediated -at least in part- by COX-2-derived prostanoids (e.g., prostaglandin [PG]E₂) driving the expression of multiple tumor-promoting cytokines and growth factors in the local tumor microenvironment, while preventing type I immunity and other anti-tumor immune effector pathways (Zelenay et al., 2015); ii) the fact that the apparent chemoprevention, as reported by many observational studies,
was a class effect of NSAIDs (Thun et al., 2002); iii) the convincing demonstration of a protective
effect of selective COX-2 inhibitors against colorectal adenoma recurrence, as provided by two
adequately powered, placebo-controlled RCTs (Baron et al., 2006; Bertagnolli et al., 2006).

Findings that do not fit into this hypothesis include: i) the chemopreventive effect of aspirin,
a drug with a 20-min half-life, against colorectal adenoma recurrence or cancer at doses as low as
81 mg given once daily or 100 mg every other day, with no greater protection afforded by a 4-fold
higher dose (Baron et al., 2003); ii) the apparent lack of a dose-effect in the post-hoc analyses of
aspirin cardiovascular RCTs, when indirectly comparing the long-term risk of developing colorectal
cancer associated with daily doses as low as 75 mg with doses as high as 1,200 mg (Flossmann and
Rothwell, 2007; Rothwell et al., 2010). If COX-2 inhibition is required for aspirin-mediated
chemoprevention, one would expect clear evidence for a dose-dependent effect, as characterized
for aspirin-induced inhibition of PGI² biosynthesis (FitzGerald et al., 1983), the main product of
COX-2 activity in the human vasculature (McAdam et al., 1999).

An alternative mechanism of action has been proposed to explain the chemopreventive
effect of low-dose aspirin, i.e., irreversible platelet COX-1 suppression (Patrignani and Patrono, 2016).
Platelet activation and the resulting release of soluble platelet
factors are important determinants of the balance between tissue damage and tissue repair
(Gawaz and Vogel, 2013). Physiologic platelet activation at sites of gastrointestinal mucosal injury
is responsible for the release of a variety of pro-inflammatory and pro-angiogenic autacoids,
including PGE₂ and TXA₂, that may induce localized and transient COX-2 expression in adjacent
nucleated cells to promote tissue repair and mucosal healing (Patrignani et al, 2001). Pharmacologic
interference with this repair mechanism may explain the two-fold increased risk of gastrointestinal
bleeding from pre-existing lesions associated with use of low-dose aspirin (and other antiplatelet
agents) and selective COX-2 inhibitors, despite endoscopic evidence of limited (new) gastroduodenal injury induced by these agents (Laine et al., 2004).

We have hypothesized that abnormal and persistent stimuli to platelet activation at sites of injured colorectal mucosa may lead to a chain of unrestrained cellular events mediated by enhanced COX-2 expression, and contribute to increased risk of sporadic adenoma formation, as depicted in Figure 3 (Patrono et al., 2001; Patrignani and Patrono, 2016). This hypothesis may explain the similar chemopreventive effect of low-dose aspirin and selective COX-2 inhibitors against sporadic colorectal adenoma recurrence, as reviewed above.

The contribution of COX-1-dependent platelet activation to the early stages of colorectal carcinogenesis is not at odds with the importance of COX-2-driven inflammation in this setting, as indicated by the demonstration that platelet-specific deletion of COX-1 ameliorates dextran sulfate sodium-induced colitis in mice (Sacco et al., 2019), and experimental evidence that selective deletion of COX-1 in megakaryocytes/platelets can mitigate early events of intestinal tumorigenesis by restraining COX-2 induction (Bruno et al., 2022).

Interestingly, antiplatelet therapy with aspirin/clopidogrel or ticagrelor but not NSAID treatment with sulindac prevented non-alcoholic steatohepatitis and subsequent hepatocellular carcinoma development in mice (Malehmir et al., 2019). Platelet GPIbα was identified as a mediator and potential interventional target for non-alcoholic steatohepatitis and subsequent liver cancer (Malehmir et al., 2019).

Several experimental findings suggest that targeting platelet activation with aspirin, but also other antiplatelet agents including P2Y<sub>12</sub> blockers, may represent an effective strategy to prevent the development of cancer metastases (Patrignani and Patrono, 2018). Selective deletion of P2Y<sub>12</sub> in the murine platelet progenitors and crossing with Apc<sup>Min/+</sup> mice, with a similar approach as used by Bruno et al (2022) to probe the role of platelet COX-1 in intestinal polyposis, may assess the
potential participation of ADP-dependent platelet activation in colorectal tumorigenesis. Furthermore, evidence that cancer diverts the tissue-repairing and hemostatic functions of platelets to suppress antitumor T-cell immunity (Rachidi et al., 2017) reinforces the rationale for antiplatelet trials in the adjuvant setting (see below).

Finally, it should be mentioned that the gut microbiome might play a role in mediating, at least in part, the anticancer effects of aspirin (Drew and Chan, 2021). Thus, experimental studies show that certain gut microbes promote a chronic inflammatory response leading to COX-2 induction and enhanced PGE₂ biosynthesis (Drew and Chan, 2021). Moreover, there appears to be mutual interaction between gut microbes degrading aspirin and limiting its systemic bioavailability and aspirin modifying the composition of the gut microbiome towards diminished abundance of genera favoring colorectal tumorigenesis (Drew and Chan, 2021).

8. Ongoing studies

A number of RCTs of aspirin are currently testing its efficacy and safety in early-stage solid tumors for the prevention of recurrence and metastasis after primary treatment with curative intent. The Add-Aspirin trial represents the largest and most comprehensive test of its chemopreventive properties in the adjuvant setting (Joharatnam-Hogan et al, 2019). The Add-Aspirin protocol includes four individually powered phase-3 RCTs evaluating the effect of daily aspirin on recurrence and survival after radical cancer therapy in the following tumor cohorts: gastro-esophageal, colorectal, breast, and prostate cancer. An open-label run-in phase (aspirin 100 mg daily for 8 weeks) precedes double-blind randomization (for participants aged under 75 years, aspirin 300 mg, aspirin 100 mg, or matched placebo in a 1:1:1 ratio; for patients aged 75 years or older, aspirin 100 mg or matched placebo in a 2:1 ratio) (Joharatnam-Hogan et al, 2019).
Approximately 8,000 patients have been recruited and three of the four cohorts will be completed in 2023. Primary outcome data are expected in 2025-2027 (Prof. Ruth Langley, personal communication).

Persistently increased TXA₂-dependent platelet activation was detected after radical cancer therapy in a sub-study of 716 patients from the four tumor cohorts of the Add-Aspirin trial, though most marked in those with colorectal and gastro-esophageal cancer where epidemiological evidence for the anti-cancer effects of aspirin is strongest. Aspirin 100 mg daily was sufficient to suppress platelet activation following radical therapy, with no further reduction in TXA₂ biosynthesis following 3-month treatment with 300 mg daily (Joharatnam-Hogan, Hatem et al., 2023). Integration of these findings with outcome data from the five-year follow-up of the Add-Aspirin participants will allow a mechanistic interpretation of any site-specific and/or dose-dependent effects of the intervention.

There are also several other groups evaluating aspirin in the adjuvant setting, through 7 RCTs in over 6,700 patients with colorectal cancer, and they have come together to form the Adjuvant Aspirin Trialists Collaboration – Colorectal Trials (Figure 4).

Finally, to address the question of the optimal dose of aspirin for patients with the Lynch syndrome, CAPP3 was designed as a randomized non-inferiority trial of aspirin 100 mg and 300 mg compared with the dose of 600 mg daily used in CAPP-2, administered in a blinded fashion for 2 years followed by open-label treatment for a further 3 years and assessment of Lynch syndrome cancers annually thereafter (Burn et al., 2020).

9. Conclusion
Considerable progress has been made during the past 20 years in mechanistic understanding of the participation of COX-isozymes in colorectal carcinogenesis, as well as in the clinical assessment of the chemopreventive properties of COX-inhibitors. While there was hope that the improved gastrointestinal safety of selective COX-2 inhibitors might deliver the appropriate tool for long-term cancer prevention and treatment, their mechanism-based cardiovascular toxicity (Patrono and Baigent, 2014) has severely restricted this possibility. On the contrary, aspirin has emerged as a promising anti-cancer strategy, due to its well-known safety profile and cardioprotective properties at low doses (Patrono, 2015). The current status of prospective, randomized assessment of its chemopreventive properties at different stages of colorectal carcinogenesis is summarized in Figure 5.

Within the next 5 years, all of the ongoing colorectal cancer RCTs listed in Figure 4 should be completed. In addition to providing statistically robust information on the efficacy and safety of low-dose aspirin in the adjuvant setting of this common cancer, an IPD meta-analysis of the 7 RCTs will provide an interesting opportunity for clinically oriented sub-group analyses and mechanistic considerations. Whether a chemopreventive benefit extends to non-digestive tract cancers will be answered by the Add-Aspirin trial, and whether a chemopreventive benefit of aspirin requires any higher dose than 100 mg daily will be addressed by the randomized dose comparisons of both the Add-Aspirin and CAPP3 trials.

Should these RCTs and additional experimental studies provide supportive evidence for the antiplatelet hypothesis of its mechanism of action, a new wave of phase-2 trials of other antiplatelet agents (and their combination) in cancer patients may provide further insight into the role of platelet activation in colorectal carcinogenesis and the rationale for phase-3 RCTs of dual antiplatelet therapy in this setting.
10. Data Availability Statement

This article contains no datasets generated or analyzed during the current study.

11. References


12. Footnotes

*This work was supported by a Catalyst Grant from Cancer Research UK to the AsCaP Consortium.

**During the past 5 years, I received consultant and speaker fees from AbbVie, Acticor Biotech, Amgen, Bayer, Eli Lilly, GlaxoSmithKline, Tremeau, and Zambon. I chaired the Scientific Advisory Board of the International Aspirin Foundation.

During the past 20 years, I received grant support for investigator-initiated research from the Italian Drug Agency (AIFA), Bayer AG, Cancer Research UK, and the European Commission, FP6 and FP7 Programmes.

13. Authorship contribution

Participated in research design: Patrono C

Wrote the manuscript: Patrono C
14. Figure Legends

Figure 1. Forest plot of pooled relative risk (RR) of non-digestive tract cancers, and corresponding 95% confidence interval (CI), for regular aspirin use vs non-use. Data are from Santucci et al, 2021.

Figure 2. Effect of aspirin on long-term risk of colorectal cancer, analyzed post-hoc (left panels) or prospectively (right panels). The effect of aspirin (75-600 mg daily) assignment (red line) versus control (blue line) on subsequent incidence of colorectal cancer is shown for the following randomized trials: upper left panel, British Doctors Aspirin Trial (BDAT) and UK-Transient Ischaemic Attack (TIA) trial; lower left panel, Thrombosis Prevention Trial (TPT) and Swedish Aspirin Low Dose Trial (SALT); upper right panel, time to first colorectal cancer in all Cancer Prevention Programme (CaPP)2 study participants (n=861) followed up for 10 years and for 20 years in England, Finland, and Wales; lower right panel, prospective long-term follow-up of 39,876
women aged 45 and over in the Women’s Health Study (WHS), 33,682 of whom continued observational follow-up, with p-value from log-rank test.


Figure 3. The potential role of platelet activation in the early stage of colorectal carcinogenesis. In the first stages of intestinal tumorigenesis, platelets may play a key role, since they are activated in response to intestinal mucosal injury and participate in tissue repair. However, when platelet activation is not restrained in time and space, the same mechanism may contribute to the induction of several signaling pathways through paracrine soluble mediators, such as thromboxane (TX)A2 and prostaglandin (PG)E2, growth factors and inflammatory cytokines, in turn inducing COX-2 expression in adjacent nucleated cells and an eicosanoid amplification loop promoting cell proliferation and angiogenesis. A sequential involvement of COX-1 (in platelets) and COX-2 (in various nucleated cells) in the early events leading to the transformation of an apparently normal intestinal mucosa into an adenomatous lesion would explain the similar protective effect of low-dose aspirin and selective COX-2 inhibitors in reducing the recurrence rate of a sporadic colorectal adenoma over the first 3 years of treatment, and protecting against cancer development over 5-10 years.

This working hypothesis was first articulated by Patrono et al (2001) and further developed by Patrignani & Patrono (2016; 2018).

Figure 4. Ongoing clinical trials evaluating aspirin in the adjuvant setting of patients with colorectal cancer. The graph shows recruitment periods, anticipated follow-up and times of the primary
analysis. Some trials (shown in red in the upper part of the figure) are only recruiting participants whose cancer has a mutation in the PIK3CA gene (PIK3CA stratified).

Courtesy of the Adjuvant Aspirin Trialists Collaboration – Colorectal Trials, co-ordinated by the MA Group of the MRC Clinical Trial Unit (Prof. Ruth Langley).

Figure 5. Stages of colorectal carcinogenesis and aspirin chemopreventive effects. The figure depicts the three clinical settings in which aspirin and other cyclooxygenase inhibitors have been or are being evaluated by randomized clinical trials. See text for individual trial details.

CR, colorectal; CRC, colorectal cancer; Lynch S., Lynch syndrome; RCT, randomized controlled trial.
Figure 1

Cancer Location | RR (95% CI)
--- | ---
Lung | 0.88 (0.79-0.98)
Breast | 0.90 (0.85-0.95)
Endometrial | 0.91 (0.84-0.98)
Ovarian | 0.91 (0.85-0.97)
Prostate | 0.93 (0.89-0.96)
Bladder | 1.03 (0.99-1.08)
Kidney | 1.06 (0.96-1.16)
Injured Colorectal Mucosa

Aspirin

Pro-angiogenic

TXA₂

PDGF

TGFβ

 activates endothelial cells

Endothelial cells

COX-2 induction

PGE₂

Angiogenesis

↑

Activates Stromal cells

Stromal cells

COX-2 induction

PGE₂

↓ Apoptosis ↑ Cellular proliferation

Sporadic Colorectal Adenoma