Effect of Low-dose and Standard-dose Aspirin on PGE_2 Biosynthesis Among Individuals with Colorectal Adenomas: A Randomized Clinical Trial

David A. Drew[1,2,3], Madeline M. Schuck[1,2], Marina V. Magicheva-Gupta[1,2], Kathleen O. Stewart[2], Katherine K. Gilpin[1,2], Patrick Miller[1,2], Melanie P. Parziale[1,2], Emily N. Pond[1,2], Oliver Takacsi-Nagy[1,2], Dylan C. Zerjav[1,2], Samantha M. Chin[1,2], Jennifer Mackinnon Krems[1,2], Dana Meixell[1,2], Amit D. Joshi[1,2], Wenjie Ma[1,2], Francis P. Colizzo[2], Peter J. Carolan[2], Norman S. Nishioka[2], Kyle Staller[1,2], James M. Richter[2], Hamed Khalili[1,2], Manish K. Gala[1,2], John J. Garber[2], Daniel C. Chung[2], Joseph C. Yarze[2], Lawrence Zukerberg[4], Giovanna Petrucci[5], Bianca Rocca[5], Carlo Patrono[5], Ginger L. Milne[6], Molin Wang[3,7,8], and Andrew T. Chan[1,2,8]

ABSTRACT

Low-dose aspirin is recommended by the U.S. Preventive Services Task Force for primary prevention of colorectal cancer in certain individuals. However, broader implementation will require improved precision prevention approaches to identify those most likely to benefit. The major urinary metabolite of PGE_2, 11α-hydroxy-9,15-dioxo-2,3,4,5-tetranor-prostane-1,20-dioic acid (PGE-M), is a biomarker for colorectal cancer risk, but it is unknown whether PGE-M is modifiable by aspirin in individuals at risk for colorectal cancer. Adults ($N = 180$) who recently underwent adenoma resection and did not regularly use aspirin or NSAIDs were recruited to a double-blind, placebo-controlled, randomized trial of aspirin at 81 or 325 mg/day for 8–12 weeks. The primary outcome was postintervention change in urinary PGE-M as measured by LC/MS. A total of 169 participants provided paired urine samples for analysis. Baseline PGE-M excretion was 15.9 ± 14.6 (mean ± S.D, ng/mg creatinine). Aspirin significantly reduced PGE-M excretion (−4.7 ± 14.8) compared with no decrease (0.8 ± 11.8) in the placebo group ($P = 0.015$; mean duration of treatment = 68.9 days). Aspirin significantly reduced PGE-M levels in participants receiving either 81 (−15%; $P = 0.018$) or 325 mg/day (−28%; $P < 0.0001$) compared with placebo. In 40% and 50% of the individuals randomized to 81 or 325 mg/day aspirin, respectively, PGE-M reduction reached a threshold expected to prevent recurrence in 10% of individuals. These results support that aspirin significantly reduces elevated levels of PGE-M in those at increased colorectal cancer risk to levels consistent with lower risk for recurrent neoplasia and underscore the potential utility of PGE-M as a precision chemoprevention biomarker. The ASPIRED trial is registered as NCT02394769.

Introduction

The 2016 U.S. Preventive Services Task Force (USPSTF) guidelines recommend the use of aspirin for primary prevention of cardiovascular disease and colorectal cancer in individuals ages 50–59 years with a greater than 10% 10-year risk of cardiovascular disease (1, 2). However, concerns remain about aspirin's potential side effects (e.g., bleeding), a lack of data in specific age groups, and the need to clarify aspirin's chemopreventive mechanisms to improve personalized treatment (1, 2). While recommendations based on age and conditioned on cardiovascular disease risk broadly identify patient populations with a higher probability for a net benefit, they lack precision, particularly related to colorectal cancer risk or potential for efficacy. We have previously proposed a paradigm that leverages established biomarkers for colorectal cancer risk, especially those that are related to aspirin's anticancer mode of action, to refine efficacy biomarkers and improve precision chemoprevention strategies (3).

As an inhibitor of the cyclooxygenase (COX) activity of prostaglandin (PG)H-synthase-1 and 2 (PTGS-1/-2, or

[1]Clinical & Translational Epidemiology Unit, Department of Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts. [2]Division of Gastroenterology, Department of Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts. [3]Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, Massachusetts. [4]Department of Pathology, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts. [5]Institute of Pharmacology, Catholic University School of Medicine and IRCCS Fondzione Policlinico Gemielli, Rome, Italy. [6]Division of Clinical Pharmacology, Vanderbilt University Medical Center, Nashville, Tennessee. [7]Department of Biostatistics, Harvard T.H. Chan School of Public Health, Boston, Massachusetts. [8]Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts.

Note: Supplementary data for this article are available at Cancer Prevention Research Online (http://cancerprevres.aacrjournals.org/).

Corresponding Author: Andrew T. Chan, Massachusetts General Hospital, GRJ-825C, 55 Fruit Street, Boston, MA 02114. Phone: 617-726-3212; Fax: 617-724-6832; E-mail: achan@mgh.harvard.edu

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COX-1/-2), aspirin blocks conversion of arachidonic acid to PGH_2 and influences a number of downstream pathways to confer aspirin's anti-inflammatory and antiplatelet effects (3). Upregulation of COX expression and dysregulated conversion of arachidonic acid into bioactive PGs, the most abundant of which is PGE_2 (4, 5), is observed in many tumor types, including colorectal cancer (3). However, despite the mechanistic links, reliable measurement of circulating PGE_2 for biomarker development is not feasible (6, 7). Instead, prior studies aimed at understanding the relationship between PGs and cancer have focused on urinary 11α-hydroxy-9,15-dioxo-2,3,4,5-tetranor-prostane-1,20-dioic acid (PGE-M), a major enzymatic metabolite reflecting *in vivo* PGE_2 biosynthesis. These prior studies have demonstrated that PGE-M is associated with an increased risk of colorectal neoplasia (8–10) and other cancer types, including pancreas (11, 12), stomach (13, 14), lung (15), and breast (16, 17), but not ovary (18). We previously demonstrated in a prospective study that elevated prediagnostic PGE-M levels are associated with an increased risk of future advanced colorectal adenomas. Furthermore, protection associated with regular aspirin/NSAID use was only observed among those with elevated prediagnostic PGE-M (8). Thus, PGE-M may be useful as a precision chemoprevention marker. Previous studies have shown that use of aspirin and other NSAIDs are cross-sectionally associated with lower PGE-M levels (8, 9, 19). However, it is unknown whether aspirin modifies baseline PGE-M levels in the target, at-risk population, and whether such a reduction is dose dependent. To address this knowledge gap, we conducted the "ASPirin Intervention for the REDuction of colorectal cancer risk" (ASPIRED) randomized, placebo-controlled trial (20) to assess the effects of aspirin at 81 and 325 mg/day on urinary PGE-M in individuals at elevated risk for colorectal cancer.

Materials and Methods

Clinical trial population and design

This study was a single-site, randomized, double-blind, placebo-controlled trial of 81 or 325 mg/day aspirin in adults ages 18–80 years old (www.clinicaltrials.gov; NCT02394769). The trial was activated in July 2015 and completed enrollment in February 2019. The detailed trial protocol has been previously published and is available via open access (20). Participants were drawn from the patient population of the Massachusetts General Hospital (MGH, Boston, MA) who had undergone a screening or surveillance colonoscopy with a pathologically confirmed diagnosis of at least one colorectal adenomatous polyp (including sessile serrated adenoma, but excluding hyperplastic polyp) within 9 months of study enrollment. All subjects provided written consent and the protocol was approved by the Dana–Farber/Harvard Cancer Center Institutional Review Board (Boston, MA, DF/HCC #14-496).

Potential participants were identified using the MGH Pathology Natural Language Search tool to generate monthly reports of individuals with adenoma that were subsequently confirmed by a member of the study team using the corresponding pathology report. The individual's treating physician invited individuals with confirmed adenoma by mail. Interested participants were recruited and eligibility was confirmed during a phone interview. Eligible participants were those that had not taken aspirin at any dose in the last 6 months, presented with an Eastern Cooperative Oncology Group performance status ≤2, and had the ability to understand and the willingness to sign a written, informed consent document. Individuals were excluded if they used any non-aspirin NSAID at any dose three or more times per week during the 2 months prior to randomization; were receiving any other investigational agents; had any prior diagnosis of gastrointestinal cancer (including esophageal, gastric, small intestine, colon, and pancreatic), or any diagnosis of other cancers (with the exception of nonmelanoma skin) in which there had been an active treatment within the 3 years prior to randomization; had a history of inflammatory bowel disease, liver, or kidney disease; had a history of aspirin intolerance or known allergic reaction to compounds of similar chemical or biological composition to aspirin; had a history of bleeding diathesis, peptic ulcer or gastrointestinal bleed, endoscopic complications, or contraindication to colonoscopy; were taking any anticoagulant agent (e.g., warfarin) or antiplatelet agent (e.g., clopidogrel); received a prior diagnosis of familial adenomatous polyposis or hereditary nonpolyposis colorectal cancer (Lynch Syndrome); had any adenoma that was not completely removed during the previous colonoscopy; were pregnant or breastfeeding; were unable to swallow pills; had an uncontrolled intercurrent illness that would limit compliance with study requirements; and were unable or unwilling to abstain from nonprotocol use of aspirin or NSAIDs or to provide blood, urine, or stool samples or colon biopsies during the study. Participants were educated via a post-card sized handout of the brand and generic names of NSAIDs that would make them ineligible from continued participation in the study. Those that reported nonstudy NSAID use during the study period were removed from the study and an exit interview was performed at that time. The informed consent process was performed by a study physician.

Study intervention

Participants ($N = 180$) were assigned to three arms that consisted of placebo (lactose), 81 mg, or 325 mg of generic, noncoated, aspirin (acetylsalicylic acid) in a 1:1:1 ratio using block random assignment generated by the study statistician (M. Wang). Aspirin capsules were prepared by the MGH Research Pharmacy to be indistinguishable from placebo and also contained lactose filler. Participants were provided with 84 blinded capsules (12-week supply) at the baseline visit and instructed to take one capsule after the clinical visit with food and water, to be repeated daily until returning for the follow-up visit. All participants, providers, and study staff were blinded to assignments. Missed doses were not made up and reported to study staff during weekly phone calls. Adherence was measured

by pill count, and additionally assessed biochemically (21) by measuring thromboxane (TX) levels in a subset of patients.

Study visits and assessments

Prior to randomization, participants attended a baseline clinical study visit at the MGH Gastrointestinal Unit where they provided biospecimens, including urine and blood samples, and underwent an unsedated flexible sigmoidoscopy without bowel preparation for stool and biopsy collection. The follow-up (posttreatment) visit mirrored the baseline visit with identical sample collection and occurred at least 8 weeks (56 days), but no more than 12 weeks (84 days), after the baseline visit. All metadata were derived by clinical assessment, self-reported questionnaire, and/or abstraction of the electronic medical record.

Endpoint ascertainment

The predefined primary outcome was the effect of aspirin at 81 or 325 mg/day on urinary PGE-M. The Eicosanoid Core Laboratory at Vanderbilt University (Nashville, TN) measured PGE-M levels in baseline/pretreatment and posttreatment urine samples using LC/MS as described previously (18). For secondary exploratory outcomes, concentrations of the urinary TX metabolite, 11-dehydrothromboxane B_2 (TXM), and serum TXB_2 and PGE_2, were quantified on samples sent to the Institute of Pharmacology at Catholic University School of Medicine (Rome, Italy), using ELISA as described previously (22–27). Additional details are provided in the Supplementary Data.

Sample size

The trial sample size was powered to detect an effect on urinary PGE-M between the placebo group and the two aspirin groups combined, regardless of compliance with the study treatment (intent-to-treat). On the basis of prior studies (7, 8, 15), we assumed a SD of 5.0 for a single measurement of PGE-M and an intraclass correlation of 0.1. With 45 participants in the placebo group and 90 participants in the combined aspirin groups, we expected 90% (80%) power to detect a mean change of PGE-M level in the aspirin group of 4.0 (3.5) ng/mg, compared with no change in the placebo group, assuming a type I error rate of 0.05. This minimum detectable difference in mean change was consistent with the difference in the median level of PGE-M among individuals at high risk for adenoma compared with low risk (8). To account for drop out of up to approximately 20% participants, we conservatively enrolled 60 participants in each group.

Statistical analysis

Baseline characteristics of subjects were compared between treatment arms by using Fisher's exact test for categorical variables and one-way ANOVA and unpaired two sample *t* tests for continuous variables. Intention-to-treat analyses comparing the effect of aspirin treatment (grouped) on posttreatment change in PGE-M (ΔPGE-M) compared with the change in the placebo group using an unpaired two-sample *t* test was performed to test the prespecified primary outcome. For subgroup analyses according to dose, a one-way ANOVA was performed followed by individual unpaired two-sample *t* tests between treatment arms. For subsequent analyses, assumptions for normality (Gaussian distribution) were checked using the Shapiro–Wilk test. Because normality assumptions were not met for urinary TXM or serum TXB_2 and PGE_2, nonparametric tests (Kruskal–Wallis and/or Mann–Whitney) were performed for these analyses. Spearman correlations were performed to compare continuous measures from urine and serum at baseline or the change in levels of related metabolites. In secondary analyses, we examined whether sociodemographic, lifestyle, and medical history factors were independently associated with change in urinary PGE-M levels (ΔPGE-M) using general linear models where baseline PGE-M or ΔPGE-M (posttreatment–baseline) was modeled as the outcome and covariates were included in the model. To assess whether these factors modified treatment effects on ΔPGE-M, we included a cross-product term for the variable and treatment assignment in the models to assess for multiplicative interaction while adjusting for baseline levels of PGE-M. All statistical tests were two-sided and considered significant using an α-threshold < 0.05, except where otherwise noted, and performed using SAS (v.9.4) or Prism8 (GraphPad). All authors had access to the study data and have reviewed and approved the final article.

Results

The derivation of the final trial cohort from those screened and recruited to the study is shown in **Fig. 1**. Baseline characteristics are presented in **Table 1**. No significant differences were observed between treatment groups (all $P > 0.05$). As per protocol, six participants were removed from the study early due to reported NSAID use during the intervention period; three withdrew prior to the final visit, and two were lost to follow-up. No serious adverse events were reported. Minor adverse events and participant complaints documented during the study were limited and occurred at similar frequency across arms (Supplementary Table S1). This resulted in 169 participants with available pre- and posttreatment urine samples for analysis. No significant differences were observed among the remaining participants according to treatment arm.

Urinary levels of PGE-M at baseline (pretreatment) and posttreatment are shown in **Table 2**. Our primary outcome analysis considered all individuals with urine at both timepoints on an intention-to-treat basis per protocol without accounting for the normality of the data. In 169 subjects, the mean ± SD excretion rate at baseline was 15.9 ± 14.6 ng per mg creatinine (ng/mg cr) and did not differ significantly between arms. The primary outcome that aspirin intervention at either dose resulted in a decrease in urinary PGE-M compared with placebo ($P = 0.015$) was successfully achieved with a mean ΔPGE-M of −4.7 ± 14.8 ng/mg cr (median = −3.5 ng/mg cr). According to dose, a mean ΔPGE-M of −4.6 ± 17.7 ($P = 0.056$) was observed for 81 mg/day and −4.9 ± 11.2 ng/mg cr ($P = 0.01$) for 325 mg/day, corresponding to a mean decrease

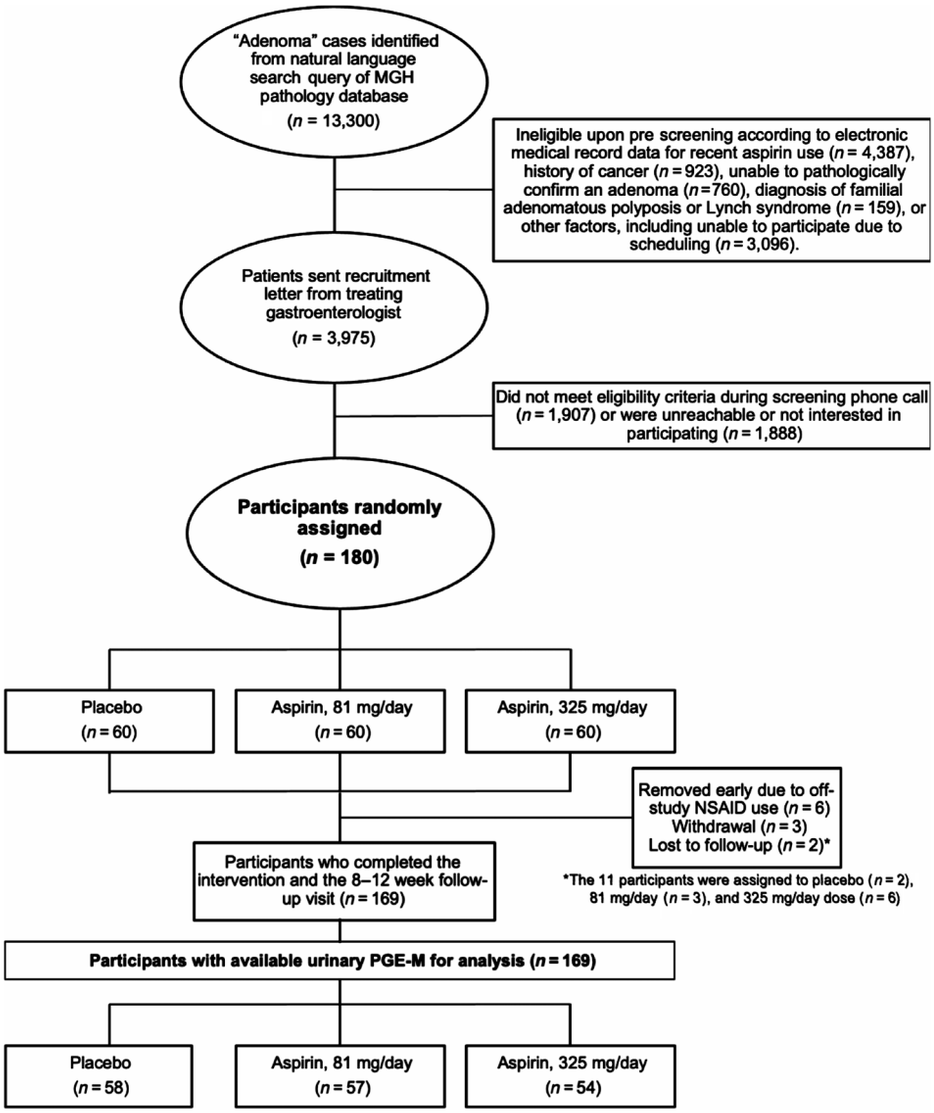

Figure 1.
ASPIRED recruitment and participant enrollment overview (CONSORT diagram).

of 15% ($P = 0.018$) and 28% ($P < 0.0001$; median decrease = 27% and 35%, respectively) after treatment. No significant difference was observed in ΔPGE-M between the aspirin groups ($P = 0.91$). The mean ΔPGE-M among the placebo group was negligible (+0.8 ng/mg cr or +8.5%).

We performed several sensitivity analyses to check the robustness of the primary outcome analysis. The data was not normally distributed; however, results were similar when using nonparametric Kruskal–Wallis and Mann–Whitney tests, with the only major difference supporting a significant decrease in urinary PGE-M between placebo and 81 mg/day arms ($P < 0.001$; Supplementary Table S2). We did observe that one individual in each of the aspirin treatment arms had abnormally high baseline urinary PGE-M. After removing these participants from the analysis, the primary outcome was not materially altered ($P = 0.018$; Supplementary Table S3). Removing these outliers attenuated the mean decrease in absolute PGE-M levels among the aspirin intervention arms, but median values and relative percent changes remained unaltered.

We considered the biological relevance of these changes in PGE-M. First, we previously reported that risk of advanced adenoma was restricted to individuals who were in the highest quartile of PGE-M concentration (Q4 median = 9.44 ng/mg cr) at baseline compared with those in lower quartiles, including quartile 3 (median = 6.28 ng/mg cr; ref. 8). Thus, a reduction of at least 33.5% in PGE-M level may be consistent with lowered risk of advanced adenoma. Calculating the population attributable risk based on these RR estimates, achieving this threshold of inhibition translates to a 10% absolute risk reduction for advanced adenomas. In ASPIRED, aspirin reduced PGE-M beyond this threshold in 40% (**Fig. 2**; $P < 0.001$; χ^2) of individuals randomized to 81 mg/day and 50.0% ($P < 0.001$; χ^2) of individuals randomized to 325 mg/day. Second, in the Aspirin/Folate Polyp Prevention Study (AFPPS), among

Table 1. Baseline characteristics of ASPIRED trial participants ($N = 180$).

	Placebo ($n = 60$)	Aspirin, 81 mg ($n = 60$)	Aspirin, 325 mg ($n = 60$)
Age, year, mean (SD)	57.1 (9.2)	56.1 (8.7)	57.5 (8.3)
Sex, *n* (%)[a]			
Female	28 (46.7)	29 (48.3)	28 (46.7)
Race, *n* (%)			
White	55 (91.7)	52 (86.7)	53 (88.3)
Black/African American	3 (5.0)	4 (6.7)	3 (5.0)
Asian	1 (1.7)	0 (0)	2 (3.3)
More than one race	0 (0)	4 (6.7)	2 (3.3)
Did not report	1 (1.7)	0 (0)	0 (0)
Ethnicity, *n* (%)			
Hispanic	2 (3.3)	2 (3.3)	1 (1.7)
Marital status, *n* (%)			
Married	40 (66.7)	39 (65.0)	37 (61.7)
Never married	6 (10.0)	12 (20.0)	11 (18.3)
Separated	2 (3.3)	0 (0.0)	1 (1.7)
Divorced	8 (13.3)	7 (11.7)	7 (11.7)
Widowed	4 (6.7)	2 (3.3)	4 (6.7)
BMI, kg/m^2, mean (SD)	26.8 (5.0)	28.4 (4.9)	27.5 (5.7)
Normal, <18.5–24.9	21 (35.0)	16 (26.7)	21 (35.0)
Overweight, 25.0–29.9	26 (43.3)	26 (43.3)	23 (38.3)
Obese, ≥30.0	13 (21.7)	18 (30.0)	16 (26.7)
Smoking status, *n* (%)			
Never	38 (63.3)	36 (60.0)	32 (53.3)
Former	18 (30.0)	20 (33.3)	19 (31.7)
Current	4 (6.7)	3 (5.0)	8 (13.3)
Missing	0 (0.0)	1 (1.7)	1 (1.7)
Alcohol consumption, *n* (%)			
Never	7 (11.7)	11 (18.3)	11 (18.3)
Rarely	14 (23.3)	16 (26.7)	18 (30.0)
1–5 times/week	29 (48.3)	24 (40.0)	23 (38.3)
Daily	10 (16.7)	8 (13.3)	6 (10.0)
More than daily	0 (0.0)	1 (1.7)	2 (3.3)
Personal cancer history, yes, *n* (%)	10 (17.0)	6 (10.0)	4 (6.8)
Family history of CRC, yes, *n* (%)	13 (21.7)	10 (16.7)	12 (20.0)
Type II diabetes, yes, *n* (%)	2 (3.4)	3 (5.0)	2 (3.3)
Menopause status ($n = 85$)[b]			
Premenopausal	3 (10.7)	9 (31.0)	5 (17.9)
Perimenopausal	4 (14.3)	1 (3.4)	2 (7.1)
Postmenopausal	20 (71.4)	17 (58.6)	18 (64.3)
Missing	1 (3.6)	2 (6.9)	3 (10.7)
History of 81 mg aspirin use, *n* (%)			
Never	55 (91.7)	50 (83.3)	53 (88.3)
Intermittently (<2 ×/week)	2 (3.3)	5 (8.3)	5 (8.3)
Regularly (>2 ×/week)	2 (3.3)	2 (3.3)	2 (3.3)
Missing	1 (1.7)	3 (5.0)	0 (0.0)
History of 325 mg aspirin use, *n* (%)			
Never	40 (66.7)	42 (70.0)	42 (70.0)
Intermittently (<2 ×/week)	17 (28.3)	15 (25.0)	17 (28.3)
Regularly (>2 ×/week)	1 (1.7)	1 (1.7)	1 (1.7)
Missing	2 (3.3)	2 (3.3)	0 (0.0)
History of NSAID use, *n* (%)			
Never	18 (30.0)	13 (21.7)	19 (31.7)
Intermittently (<2 ×/week)	31 (51.7)	36 (60.0)	32 (53.3)
Regularly (>2 ×/week)	10 (16.7)	9 (15.0)	8 (13.3)
Missing	1 (1.7)	2 (3.3)	1 (1.7)
PPI use, *n* (%)			
Current and regular	5 (8.3)	6 (10.0)	8 (13.3)
Missing	7 (11.7)	5 (8.3)	4 (6.7)
H2-blocker use, *n* (%)			
Current and regular	2 (3.3)	5 (8.3)	2 (3.3)
Missing	2 (3.3)	1 (1.7)	1 (1.7)
Antacid use, *n* (%)			
Current and regular	5 (8.3)	3 (5.0)	3 (5.0)
Missing	1 (1.7)	0 (0.0)	0 (0.0)
Statin use, *n* (%)			
Current and regular	14 (23.3)	11 (18.3)	16 (26.7)
Missing	2 (3.3)	1 (1.7)	1 (1.7)
Indication for previous endoscopy, *n* (%)			
Screening	35 (58.3)	33 (55.0)	30 (50.0)
Surveillance	15 (25.0)	13 (21.7)	16 (26.7)
Diagnostic	3 (5.0)	5 (8.3)	3 (5.0)
Other/unknown	7 (11.7)	9 (15.0)	11 (18.3)
Polyp history by location, *n* (%)			
Right	23 (38.3)	25 (41.7)	26 (43.3)
Left	24 (40.0)	14 (23.3)	17 (28.3)
Both	13 (21.7)	21 (35.0)	16 (26.7)
Unknown	0 (0.0)	0 (0.0)	1 (1.7)
Days on treatment, mean (SD)[c]	68.6 (6.5)	68.5 (7.5)	69.6 (6.9)
Pill count adherence, *n* (%)[c]			
95.0%–100%	52 (89.7)	45 (79.3)	49 (90.7)
90.0%–94.9%	3 (5.2)	9 (15.5)	3 (5.6)
80.0%–89.9%	1 (1.7)	3 (5.2)	2 (3.7)
<80.0%	1 (1.7)	0 (0.0)	0 (0.0)
Missing	1 (1.7)	0 (0.0)	0 (0.0)

Note: Baseline characteristics of subjects were compared between treatment arms by using Fisher exact test for categorical variables and one-way ANOVA and unpaired two sample *t* tests for continuous variables. Missing represents one individual who completed the study, but did not return their pill bottle to the study staff.

Abbreviation: CRC, colorectal cancer; PPI, Proton Pump Inhibitor.

[a]Biological sex at birth.

[b]Question posed only to women.

[c]Only available for individuals who completed the study and provided a pre- and posttreatment sample ($n = 169$).

participants who were randomized to aspirin for 3-years, postintervention urinary PGE-M levels below 5.34 ng/mg cr were associated with a decreased risk of any adenoma and, specifically, advanced adenoma recurrence (19). In ASPIRED, posttreatment urinary PGE-M levels were reduced below 5.34 ng/mg cr in a significantly greater proportion of individuals randomized to 81 (**Fig. 3**; 25%; $P = 0.04$, χ^2) or 325 (41%; $P = 0.0002$, χ^2) mg/day aspirin compared with placebo.

Although the randomization resulted in no clear imbalances between arms according to baseline characteristics, we examined the impact of potential confounders on change in urinary PGE-M excretion (Supplementary Table S4). Several factors were nominally associated with increased baseline PGE-M levels: widowed or never married marital status, obesity [body

Table 2. Urinary PGE-M concentration according to randomized intervention arm.

Variable	Placebo ($n = 58$)	Aspirin dose assignment: 81 mg/day ($n = 57$)	$P_{81 \text{ vs. placebo}}$	325 mg/day ($n = 54$)	$P_{325 \text{ vs. placebo}}$	$P_{\text{aspirin (grouped) vs. placebo}}$
Baseline urinary PGE-M, ng/mg cr	15.5 (12.6)	17.7 (17.1)	0.44	14.3 (13.7)	0.62	0.82
Postintervention urinary PGE-M, ng/mg cr	16.4 (15.8)	13.1 (13.4)	0.24	9.4 (7.9)	0.005	0.018
Δ urinary PGE-M, ng/mg cr	0.8 (11.8)	−4.6 (17.7)	0.056	−4.9 (11.2)	0.010	**0.015**
% change	8.5 (50.6)	−15.4 (56.7)	0.018	−28.2 (40.3)	<0.0001	0.0003

Note: The *P* value for the primary outcome comparison is in bold. Values are mean (SD) unless otherwise noted. *P* values are generated from unpaired *t* tests between groups, as noted by the subscript text, for each measure. No significant differences were observed between aspirin treatment groups (81 vs. 325 mg/day), all $P > 0.05$.

mass index (BMI) = 30 kg/m^2 or higher), type II diabetes, greater alcohol consumption, and ever history of non-aspirin NSAID use. However, none of these associations were significant after accounting for multiple hypothesis testing (Bonferroni-adjusted $\alpha < 0.00024$; $P = 0.05/21$ covariates), other than type II diabetes ($P = 0.0002$). In addition, no significant interactions were observed between any covariate and randomized assignment that modified the effect of treatment.

Among participants who completed the study, adherence measured by pill count was high with 75% of participants exhibiting 100% adherence and only one participant at <80% (77%). Adherence did not differ significantly between arms

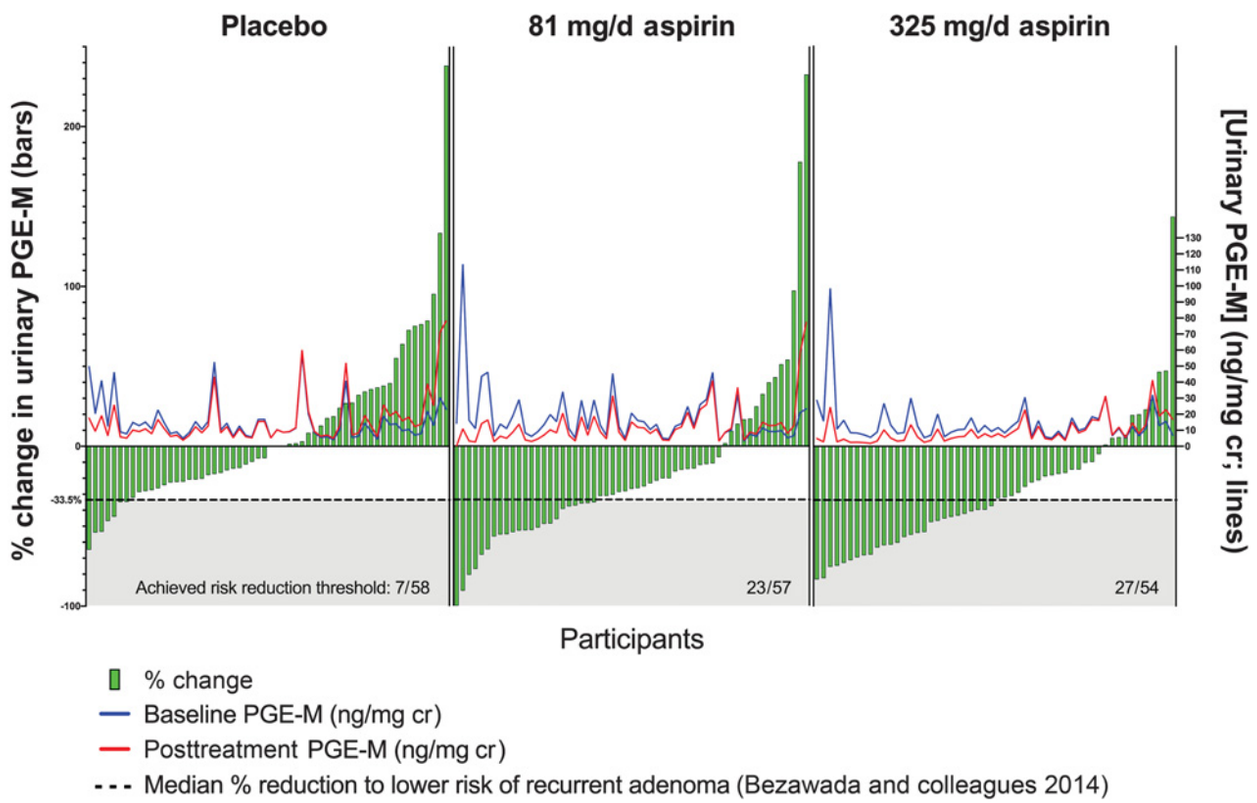

Figure 2.
Percent change in urinary PGE-M in individual ASPIRED participants according to treatment assignment in context of risk thresholds for advanced adenoma. Data from the Nurses' Health Study (Bezawada and colleagues, ref. 8) suggested that individuals with the highest quartile (Q) of PGE-M at baseline (Q4, median = 9.44 ng/mg cr) were at significantly increased risk for developing advanced adenomas compared with those in lower quartiles (Q3, median = 6.28 ng/mg cr). Thus, a reduction of 33.5% on the basis of these previously reported values may be consistent with reduced risk for advanced adenoma. The dashed line and shaded area represent the minimum threshold for change (−33.5%) at which individuals might expect a decrease in risk for recurrent neoplasia. Each green bar represents an individual's percent change in PGE-M from baseline (left, *y*-axis). Individual pre- and posttreatment PGE-M in ng/mg cr appears as the red and blue trace lines, respectively (right, *y*-axis). Aspirin intervention with 81 or 325 mg/day significantly reduced individual PGE-M levels below this threshold in a greater proportion of participants (green bars contained within gray box), 23 of 57 (40.4%) and 27 of 54 (50.0%), respectively (both $P < 0.001$; χ^2), compared with 7 of 58 (12.0%) of those randomized to placebo.

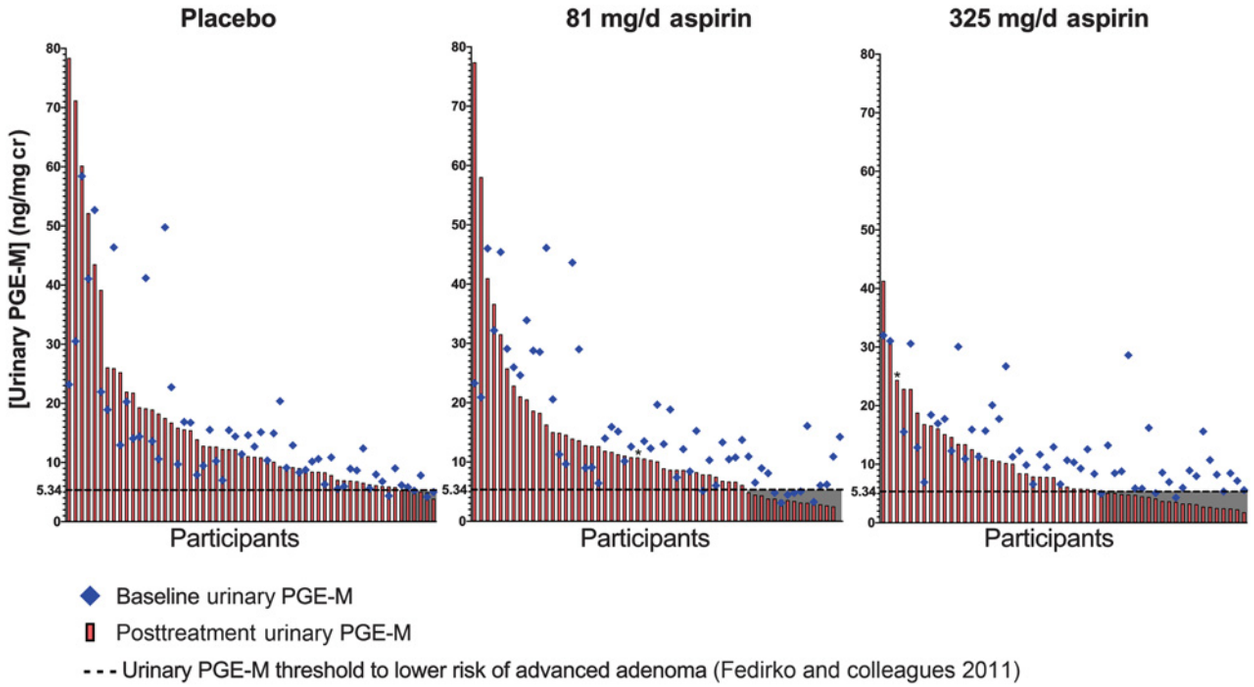

Figure 3.

Absolute change in urinary PGE-M in individual ASPIRED participants according to treatment assignment in context of risk thresholds for recurrent advanced adenoma based on the AFPPS. The AFPPS clinical trial (Fedirko and colleagues, ref. 19) reported that individuals with urinary PGE-M levels below 5.34 ng/mg cr after 3 years of aspirin treatment were at significantly reduced risk of recurrent advanced adenoma compared with individuals above this threshold. Individuals are separated by treatment arm, ranked by posttreatment PGE-M level (red bar), and plotted with pretreatment PGE-M levels (blue diamond). The dashed line and shaded area represent the minimum threshold for change (5.34 ng/mg cr) at which individuals might expect a decrease in risk for recurrent advanced neoplasia. Aspirin intervention with 81 or 325 mg/day significantly reduced PGE-M levels below this threshold in a greater proportion of individuals, 14 of 57 (24.6%; $P = 0.04$, χ^2) and 22 of 54 (40.7%; $P = 0.0002$, χ^2), respectively, compared with 6 of 58 (10.3%) of those randomized to placebo. One individual in each of the aspirin treatment arm had abnormally high pretreatment PGE-M levels [denoted by asterisk (*) in the figure]. Pretreatment PGE-M values for these individuals equaled 113.7 and 98.5 ng/mg cr in the 81 and 325 mg/day (mg/d) arms, respectively.

($P > 0.05$). Adherence was additionally checked by measuring urinary TXM in the entire cohort ($n = 169$) and serum TXB_2 among a subset ($n = 30$) who had serum collected according to optimized methods for TXB_2 assessment (Supplementary Fig. S1). At baseline, urinary TXM, an index of systemic TXA_2 biosynthesis (median: 1.7 ng/mg cr; 1.1–2.2), and serum TXB_2, a marker of platelet COX-1 activity (median: 234.7 ng/mL; 25th, 75th percentiles: 152.3, 287.5) levels were similar across arms ($P > 0.05$). Compared with placebo, 81 mg/day of aspirin reduced median TXM by 71.7% (Supplementary Fig. S1A; $P < 0.0001$) and TXB_2 by 97.9% (Supplementary Fig. S1B; $P < 0.0001$) and 325 mg/day of aspirin inhibited median TXM by 78.0% (Supplementary Fig. S1A; $P < 0.0001$) and TXB_2 by 99.8% (Supplementary Fig. S1B; $P < 0.0001$). The percent differences in these analytes between aspirin doses were significant ($P < 0.05$). Urinary TXM and serum TXB_2 were correlated at baseline (Supplementary Fig. S1C; Spearman, $r = 0.40$; $P = 0.03$) as was the post-aspirin change (Supplementary Fig. S1D; $r = 0.46$; $P = 0.03$), although these relationships have been previously demonstrated to be relatively nonlinear (28).

As an exploratory analysis, among the subset of 30 individuals with serum, we additionally tested pre- and post-treatment serum to determine whether aspirin inhibition of PGE_2 would be similarly measured in circulation. We found that baseline serum PGE_2 and urinary PGE-M concentrations were not correlated (**Fig. 4A**), perhaps reflecting the platelet versus nonplatelet source(s) of PGE_2 production. However, 81 or 325 mg/day aspirin significantly reduced serum PGE_2 compared with placebo ($P = 0.005$ and 0.0005, respectively) and no difference between treatment arms was observed (**Fig. 4B**; $P = 0.65$). The percent change in serum PGE_2 was modestly correlated to percent change in urinary PGE-M (Spearman $r = 0.40$; $P = 0.03$; **Fig. 4C**).

Discussion

Overall, standard doses of aspirin (81 or 325 mg, once daily) over 8 weeks significantly reduced pretreatment systemic PGE_2, as reflected by urinary PGE-M excretion in adults with a recent history of colorectal adenoma. Furthermore, this intervention was sufficient to reduce PGE-M excretion to levels previously associated with a reduced risk of recurrent adenoma or colorectal cancer in nearly half of the individuals randomized to aspirin. In addition, we observed profound inhibition of platelet PGE_2 and platelet

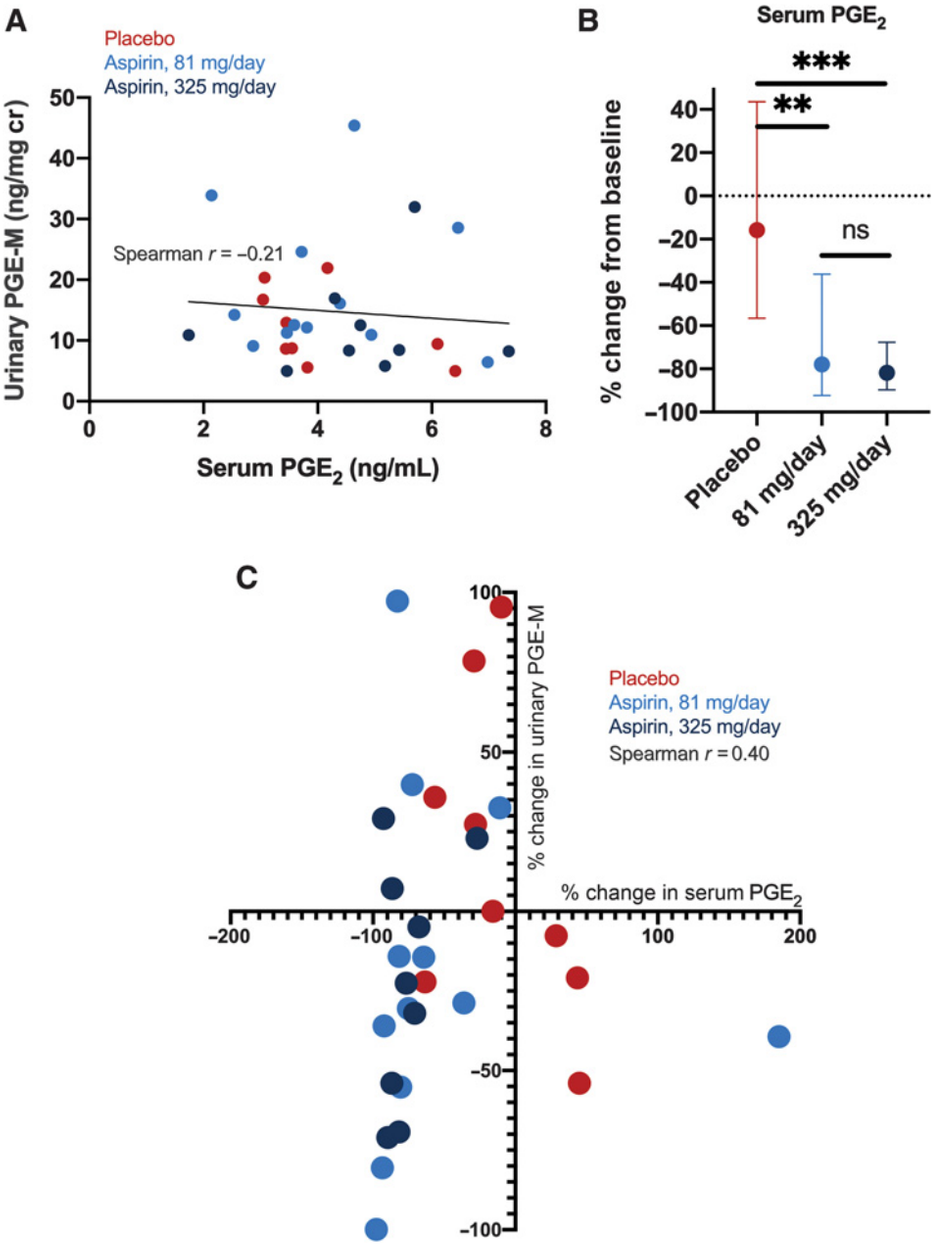

Figure 4.

PGE_2 measurement in serum of ASPIRED participants. **A,** Spearman correlation of baseline urinary PGE-M (systemic) and serum PGE_2 (circulating) demonstrates measures are not well correlated. **B,** Aspirin intervention with 81 or 325 mg/day significantly reduces serum PGE_2 from baseline compared with placebo. Mann–Whitney test; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant. **C,** The percent decrease in urinary PGE-M is modestly correlated with the percent change in serum PGE_2 following aspirin intervention. Spearman $r = 0.40$; one-tailed $P = 0.035$.

and systemic TXA_2 biosynthesis. Combined, this work demonstrates that aspirin intervention can significantly reduce elevated PGE_2 levels in patients at risk for colorectal cancer to a level consistent with reduced risk of recurrent colorectal neoplasia. These results support a causal link for aspirin's effect on PGE_2 biosynthesis as a central mechanism for its chemopreventive mode of action and that urinary PGE-M is a modifiable biomarker for colorectal cancer risk that may have utility in aspirin precision chemoprevention.

Urinary PGE-M has previously been demonstrated to be a promising biomarker to predict individual colorectal cancer risk (29), and as an efficacy marker for chemoprevention agents (30). Here, we demonstrate that aspirin reduces PGE-M in most individuals. In the AFPPS trial, which only examined urinary PGE-M only after 3 years of aspirin treatment, those randomized to 81 or 325 mg aspirin had PGE-M levels of 18% and 28% lower than those receiving placebo, respectively, which corresponds to levels 1.5–2.5 ng/mg cr lower than those receiving placebo (19). We demonstrate strikingly similar results within individuals randomized to aspirin experiencing a mean decrease of 15% and 28% from baseline for each dose, corresponding to a mean difference of approximately −4.7 ng/mg cr. Notably, our results were also consistent with the AFPPS (19), where no statistically significant differences were observed between 81 and 325 mg/day overall, but

significant inhibition, irrespective of dose, was achieved here in a much shorter timeframe. Furthermore, our results are consistent with a randomized clinical trial in current heavy smokers ($n = 54$) that demonstrated low-dose daily or intermittent aspirin reduced urinary PGE-M from baseline (31). Beyond urinary PGE-M, we also demonstrate that the change in serum PGE_2 measured by immunoassay appears correlated with the change in urinary PGE-M, offering another potential blood-based biomarker that may be conducive to implementation in clinical settings.

By examining the change from baseline urinary PGE-M levels in at-risk individuals, our study provides an opportunity to understand personalized responses to aspirin intervention. Because prior studies employed the same method for PGE-M quantitation where the concentration was normalized to an individual's urinary creatinine levels (8, 19), direct comparison of these normalized PGE-M levels across studies is reasonable, even though they are single-timepoint, cross-sectional measures. Given that our trial cohort is comprised entirely of individuals at higher risk for colorectal cancer due to their adenoma history, it is not surprising that the observed mean baseline PGE-M of 15.9 ng/mg cr places the majority of ASPIRED participants in the highest quartile of risk according to these previous reports (8, 9, 19). This finding has important implications for chemoprevention: polypectomy alone does not appear sufficient to reduce risk associated with elevated prostanoid biosynthesis in the immediate term (months after resection) for the majority of individuals diagnosed with adenoma. Biologically, this suggests that dysregulated PGE_2 biosynthesis may not be restricted to neoplastic tissue and additional intervention may be required to suppress PG-mediated carcinogenesis. Future studies may be able to distinguish the major cellular source of PGE_2, which could include platelets, stromal cells, or colorectal epithelium.

Although aspirin reduced PGE-M in the majority of individuals, we observed a decrease to levels consistent with reduced risk of recurrent neoplasia in approximately half of the individuals randomized to aspirin. While 81 mg/day was sufficient to achieve a nearly 34% reduction of PGE-M in approximately 40% of individuals randomized to aspirin, more individuals reached this threshold in the 325 mg/day arm. This is even more apparent when considering the higher threshold of decreasing levels below 5.34 ng/mg cr as a benchmark for reduced risk on the basis of the AFPPS findings (19), where nearly twice as many individuals achieved the threshold after treatment with standard dose compared with low dose. Therefore, while we did not observe a significant difference in urinary PGE-M levels between aspirin doses, higher doses may be more effective in achieving a response in individuals who do not respond to low-dose aspirin. Given the interindividual variability of prostanoid inhibition we observed, multiple timepoints or longer intervention may be required to better disentangle individual responses. Moreover, future studies may consider prioritizing percent change of urinary PGE-M from baseline over absolute change to more stably account for possible sources of variation. Nonetheless, this underscores the potential clinical utility for a precision prevention approach where flux in PGE-M levels could be used to identify individuals for whom aspirin is showing effects, even after a short burst intervention, and would likely benefit from continued use.

Conversely, this finding also highlights a subset of individuals that may be nonresponsive to aspirin. Approximately 20%–25% of those randomized to 81 or 325 mg/day experienced no inhibition or even an increase in PGE-M from baseline. In contrast, all but one participant experienced a strong reduction of TXM or TXB_2 irrespective of dose. This may reflect the contribution of constitutive expression of COX-2 from extraintestinal sites of PGE_2 biosynthesis (e.g., kidney or brain) that may require higher doses of aspirin or more frequent dosing to sustain suppression. Therefore, there may also be clinical utility for a precision prevention approach where flux in PGE-M levels may also be used to tailor dose and duration recommendations or identify individuals who might not derive any chemoprotection, and, thus, for whom the harms associated with aspirin use might outweigh potential benefits.

Future studies should examine whether more individuals experience reduction in PGE-M below risk thresholds when provided higher doses or over longer treatment periods while closely monitoring for potential risks. Observational data supports that the chemopreventive effects for aspirin are most fully appreciated after regular use of aspirin for 5–10 years (32, 33), such that future trials with longer term follow-up might vary the intervention period to clarify the ideal length and dose of intervention that translates into sustained inhibition of PGE-M and reduced risk of recurrent neoplasia. Similarly, use of baseline PGE-M might be used to identify participants as high, average, or low risk patients in context of existing risk markers (e.g., adenoma clinical and histopathologic features) prior to randomization so as to test whether PGE-M can be used as a sensitive risk stratification biomarker.

We did observe that several factors, including elevated BMI, type II diabetes, and heavy alcohol consumption, were marginally associated with baseline higher urinary PGE-M levels and may contribute to the observed interindividual variation. These factors have each independently been associated with colorectal cancer risk (34–40). BMI- and obesity-related comorbidities are of particular interest considering the potential impact on aspirin bioavailability (41, 42). Given that individuals with the highest levels of PGE-M at baseline appear to derive the greatest benefit from aspirin from prospective studies (8), these data suggest that individuals with obesity, type II diabetes, and/or consume a heavy amount of alcohol may represent populations to specifically target for aspirin following adenoma resection. While no significant interactions were observed here, larger studies should examine findings in context of these risk factors. This is especially relevant because the USPSTF confined their recommendation for aspirin use in colorectal cancer primary prevention to those individuals at 10% or greater 10-year risk for cardiovascular disease (1),

which also shares these risk factors (43). As incidence of these comorbidities continues to grow, an alternative or complementary approach to precision prevention may include incorporating additional shared risk factors for colorectal cancer and cardiovascular disease, especially those that may influence eicosanoid pools, which may predict more favorable risk-benefit profiles for aspirin.

Our study has limitations. First, urinary PGE-M is a surrogate endpoint for colorectal cancer risk and is a measure of systemic PGE_2 biosynthesis. Secondary endpoint analysis, including tissue gene expression, may further elucidate colon-specific roles for PG modulation by aspirin for chemoprevention. Second, our intervention period was relatively short. However, intraindividual reduction of PGE-M observed after 8–12 weeks of daily use in ASPIRED was consistent with interindividual PGE-M reduction between placebo and treatment arms in the AFPPS after 3 years of use. A longer intervention period would have presented additional challenges related to adherence or retention. Finally, the trial cohort was predominantly white and additional studies will be required to support generalizability of the findings for other populations.

In conclusion, our results support that low-dose, daily aspirin over a short-term period is sufficient to downregulate PGE_2 biosynthesis in many at-risk individuals to levels consistent with lower risk of colorectal cancer. However, higher doses or longer durations of treatment may be necessary to achieve significant reduction in a greater proportion of individuals. Our results support the potential utility for PGE-M for identifying individuals following adenoma resection who are more likely to derive chemopreventive benefit from aspirin. We envision that urinary PGE-M may provide a paradigm for precision prevention, by which individual response can be measured and used to tailor recommendations, including whether to continue, change, or cease aspirin in a prevention setting.

Disclosure of Potential Conflicts of Interest

D.A. Drew reports grants from NIH during the conduct of the study. D. C. Zerjav reports grants from NIH during the conduct of the study. D. Meixell reports grants from NIH during the conduct of the study. K. Staller reports personal fees from Shire (speaker), Synergy (consultant), and Arena (DSMB member), and Boston Pharmaceuticals (consultant), outside the submitted work, and grants from Takeda (research support) and AstraZeneca (research support), outside the submitted work. J.M. Richter reports grants from NCI during the conduct of the study. H. Khalili reports grants from Pfizer and Takeda, and personal fees from Takeda outside the submitted work. B. Rocca reports personal fees from Novartis and grants from Bayer AG outside the submitted work. C. Patrono reports grants from Cancer Research UK (Catalyst grant) during the conduct of the study, personal fees from Bayer (consulting and lecture fees related to aspirin and cancer) outside the submitted work, has received consultant and speaker fees from Acticor Biotech, Amgen, Bayer, GlaxoSmithKline, Tremeau, and Zambon, and chairs the scientific advisory board of the International Aspirin Foundation. A.T. Chan reports grants and personal fees from Bayer Pharma AG, personal fees from Pfizer Inc., Boehringer Ingelheim, and Janssen Pharmaceuticals outside the submitted work. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

D.A. Drew: Conceptualization, resources, data curation, software, formal analysis, supervision, funding acquisition, validation, investigation, visualization, methodology, writing-original draft, project administration, writing-review and editing. **M.M. Schuck:** Data curation, supervision, methodology, project administration, writing-review and editing. **M.V. Magicheva-Gupta:** Data curation, supervision, methodology, project administration, writing-review and editing. **K.O. Stewart:** Data curation, methodology, writing-review and editing. **K.K. Gilpin:** Data curation, methodology, writing-review and editing. **P. Miller:** Data curation, methodology, writing-review and editing. **M.P. Parziale:** Data curation, methodology, writing-review and editing. **E.N. Pond:** Data curation, methodology, writing-review and editing. **O. Takacsi-Nagy:** Data curation, methodology, writing-review and editing. **D.C. Zerjav:** Data curation, methodology, writing-review and editing. **S.M. Chin:** Data curation, methodology, writing-review and editing. **J. Mackinnon Krems:** Data curation, methodology, writing-review and editing. **D. Meixell:** Data curation, methodology, writing-review and editing. **A.D. Joshi:** Conceptualization, data curation, software, formal analysis, validation, methodology, writing-review and editing. **W. Ma:** Conceptualization, data curation, software, formal analysis, validation, methodology, writing-review and editing. **F.P. Colizzo:** Methodology, writing-review and editing. **P.J. Carolan:** Methodology, writing-review and editing. **N.S. Nishioka:** Methodology, writing-review and editing. **K. Staller:** Methodology, writing-review and editing. **J.M. Richter:** Methodology, writing-review and editing. **H. Khalili:** Methodology, writing-review and editing. **M.K. Gala:** Data curation, methodology, writing-review and editing. **J.J. Garber:** Methodology, writing-review and editing. **D.C. Chung:** Methodology, writing-review and editing. **J.C. Yarze:** Methodology, writing-review and editing. **L. Zukerberg:** Methodology, writing-review and editing. **G. Petrucci:** Data curation, writing-review and editing. **B. Rocca:** Data curation, writing-review and editing. **C. Patrono:** Data curation, writing-review and editing. **G.L. Milne:** Data curation, formal analysis, methodology, writing-review and editing. **M. Wang:** Data curation, software, formal analysis, methodology, writing-review and editing. **A.T. Chan:** conceptualization, resources, data curation, formal analysis, supervision, funding acquisition, validation, investigation, visualization, methodology, writing-original draft, project administration, writing-review and editing.

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