REVIEW

Nonsteroidal Anti-inflammatory Drugs as Anticancer Agents: Mechanistic, Pharmacologic, and Clinical Issues

Michael J. Thun, S. Jane Henley, Carlo Patrono

Numerous experimental, epidemiologic, and clinical studies suggest that nonsteroidal anti-inflammatory drugs (NSAIDs), particularly the highly selective cyclooxygenase (COX)-2 inhibitors, have promise as anticancer agents. NSAIDs restore normal apoptosis in human adenomatous colorectal polyps and in various cancer cell lines that have lost adenomatous polyposis coli gene function. NSAIDs also inhibit angiogenesis in cell culture and rodent models of angiogenesis. Many epidemiologic studies have found that long-term use of NSAIDs is associated with a lower risk of colorectal cancer, adenomatous polyps, and, to some extent, other cancers. Two NSAIDs, sulindac and celecoxib, have been found to inhibit the growth of adenomatous polyps and cause regression of existing polyps in randomized trials of patients with familial adenomatous polyposis (FAP). However, unresolved questions about the safety, efficacy, optimal treatment regimen, and mechanism of action of NSAIDs currently limit their clinical application to the prevention of polyposis in FAP patients. Moreover, the development of safe and effective drugs for chemoprevention is complicated by the potential of even rare, serious toxicity to offset the benefit of treatment, particularly when the drug is administered to healthy people who have low annual risk of developing the disease for which treatment is intended. This review considers generic approaches to improve the balance between benefits and risks associated with the use of NSAIDs in chemoprevention. We critically examine the published experimental, clinical, and epidemiologic literature on NSAIDs and cancer, especially that regarding colorectal cancer, and identify strategies to overcome the various logistic and scientific barriers that impede clinical trials of NSAIDs for cancer prevention. Finally, we suggest research opportunities that may help to accelerate the future clinical application of NSAIDs for cancer prevention or treatment. [J Natl Cancer Inst 2002;94:252–66]

Several recent reviews *(1–3)* have summarized the intriguing and accumulating evidence that nonsteroidal anti-inflammatory drugs (NSAIDs) have promise as anticancer drugs. NSAIDs have been shown experimentally to stimulate apoptosis and to inhibit angiogenesis, two mechanisms that help to suppress malignant transformation and tumor growth. Numerous epidemiologic (nonrandomized) studies *(4–37)* have found that long-term users of aspirin or other NSAIDs have a lower risk of colorectal adenomatous polyps and colorectal cancer than nonusers, although one study has not *(38–40)*. Randomized clinical trials have confirmed that two NSAIDs, the prodrug sulindac *(41–43)* and the selective cyclooxygenase (COX)-2 inhibitor celecoxib *(44)*, effectively inhibit the growth of adenomatous polyps and

cause regression of existing polyps in patients with the unusual hereditary condition familial adenomatous polyposis (FAP).

Despite these positive findings, the efficacy and safety of long-term NSAID prophylaxis against colorectal or other cancers remain unproven. While some experts have proposed that there is now sufficient evidence for persons at high risk of large bowel cancer to begin taking low-dose aspirin prophylactically *(45)*, most have not. Health organizations and consensus groups have been appropriately cautious by withholding any recommendation regarding the use of NSAIDs for the prevention or treatment of cancer, except for the use of celecoxib or sulindac to suppress the growth of colorectal adenomatous polyps in patients with FAP *(46)*. Despite enthusiasm about the potential usefulness of NSAIDs, particularly the selective COX-2 inhibitors, as anticancer agents, fundamental questions remain about their safety, efficacy, mechanisms of action, optimal treatment regimens, and contraindications for preventive therapy.

Because of the formidable challenges involved in developing safe and effective drugs for chemoprevention, discussed below, there is continuing need to improve cross-disciplinary communication in planning randomized clinical trials of NSAIDs for chemoprevention. This review combines the perspectives of two epidemiologists (M. J. Thun and S. J. Henley) and a clinical pharmacologist (C. Patrono) to examine the scientific evidence underlying randomized clinical trials of NSAIDs for cancer prevention or treatment. Using the example of aspirin prophylaxis for the prevention of cardiovascular disease, we consider the delicate balance of risks and benefits that complicates primary prevention of cancer. The safety of treatment is of particular concern when large numbers of healthy people must be treated prophylactically for many years to prevent adverse events in a small percentage of those treated. We suggest generic strategies that can improve the benefit–risk balance. We critically review the published experimental, clinical, and epidemiologic evidence regarding NSAIDs and cancer, focusing particularly on colorectal cancer, and suggest research strategies and opportunities that may help to accelerate the future clinical application of NSAIDs for the prevention or treatment of cancer.

Affiliations of authors: M. J. Thun, S. J. Henley, Department of Epidemiology and Surveillance Research, American Cancer Society, Atlanta, GA; C. Patrono, Center of Excellence on Aging, Universitá degli Studi "G. D'Annunzio," Chieti, Italy.

Correspondence to: Michael J. Thun, M.D., M.S., Department of Epidemiology and Surveillance Research, American Cancer Society, National Home Office, 1599 Clifton Rd., N.E., Atlanta, GA 30329–4251 (e-mail: mthun@cancer. org).

See "Notes" following "References."

Challenges of Chemoprevention

At least two constraints make it particularly difficult to develop safe and effective drugs to prevent cancer in average- or low-risk populations. The first is the very low margin for toxicity whenever prophylactic drugs are administered over a long period of time to healthy people who have comparatively low risk of the disease being prevented. Although colorectal cancer accounts for almost 12% of all newly diagnosed cancers in the United States *(47),* the probability that an individual at average risk will develop colorectal cancer in a given year is low. On average, the cumulative probability of developing colorectal cancer from birth to age 79 years is approximately 4% in men and 3% in women; this probability increases to 5.6% in both sexes over full-life expectancy *(48).* Thus, in the general population, more than 94% of the people treated prophylactically to prevent colorectal cancer will not benefit from prophylactic treatment unless the benefits extend to other health endpoints. However, the risk of colorectal cancer is considerably higher in certain genetically susceptible subgroups. For example, estimates of lifetime risk, based on studies of high-risk families, are approximately 17% for persons with two affected first-degree relatives, 70% for individuals with genetic mutations associated with hereditary nonpolyposis colon cancer (HNPCC), and more than 95% in persons with FAP *(49).* However, these high-risk groups contribute only a small fraction of all cases of colorectal cancer.

A second constraint to developing drugs to prevent cancer in average- or low-risk populations concerns the logistic difficulty of studying cancer endpoints, especially those associated with colorectal cancer, in large-scale prevention trials. Because of the low annual risk of developing colorectal cancer in the general population, phase III cancer prevention trials must be much larger than either trials of cardiovascular disease in high-risk

populations or therapeutic trials in cancer patients already diagnosed with disease to have adequate statistical power. More than 150 clinical trials have been conducted to assess the efficacy of aspirin as an antiplatelet agent in patients at high risk of cardiovascular disease. Most of these trials enrolled fewer than 3000 patients, and follow-up ranged from 6 months to 4 years *(50,51).* Trials of similar size and duration have sufficient statistical power to assess the prevention of recurrent colorectal adenomatous polyps in high-risk groups. In contrast, trials of colorectal cancer prevention would require up to 100 times more subjects with 10–20 years of follow-up. Furthermore, trials of both adenomatous polyps and colorectal cancer prevention are logistically more difficult than prevention trials of other cancer sites because they require that study subjects undergo special examinations (i.e., sigmoidoscopy or colonoscopy) before and after the intervention. Consequently, clinical decisions about the efficacy of chemopreventive drugs will need to be based on a smaller number of phase III trials that measure surrogate endpoints in high-risk populations. Decisions about the treatment regimen to be tested in these trials must be based largely on preclinical and epidemiologic evidence, on phase I trials involving 20–80 participants, and on phase II studies of up to several hundred subjects.

Example of aspirin in the prevention of cardiovascular events. Randomized trials of aspirin in the prevention of cardiovascular disease illustrate that the safety of prophylactic treatment is influenced critically by the background risk of the population being treated *(50,52,53).* Fig. 1 shows that the cardiovascular benefit of aspirin treatment, as measured by the prevention of myocardial infarction (MI), thrombotic stroke, or death from all causes, increases in relation to the annual background risk of these events in the population. Each data point in Fig. 1 is based on the combined data only from published randomized trials of aspirin prophylaxis at less than or equal to 500 mg daily in specified populations *(50,54–56);* therefore, the 95%

Fig. 1. Cardiovascular benefit of aspirin in relation to background cardiovascular risk. The *x*-axis indicates the percentage of placebo-treated subjects experiencing an adverse vascular event each year. The *y*-axis indicates the number of adverse cardiovascular events (myocardial infarction [MI], stroke, or death from all causes) prevented per 1000 patient-months of treatment with aspirin at less than or equal to 500 mg daily *(50,54–56).* **Each point** (and 95% confidence interval [CI]) represents the combined data from all published randomized trials of aspirin prophylaxis at less than or equal to 500 mg daily in the specified population. By comparison, the mean frequency of serious bleeding complications in aspirin trials at this dose is 0.07 per 1000 patient-months of treatment (95% CI = 0.04 to 0.09), with a maximum frequency of 0.65. TIA = transient ischemic attack.
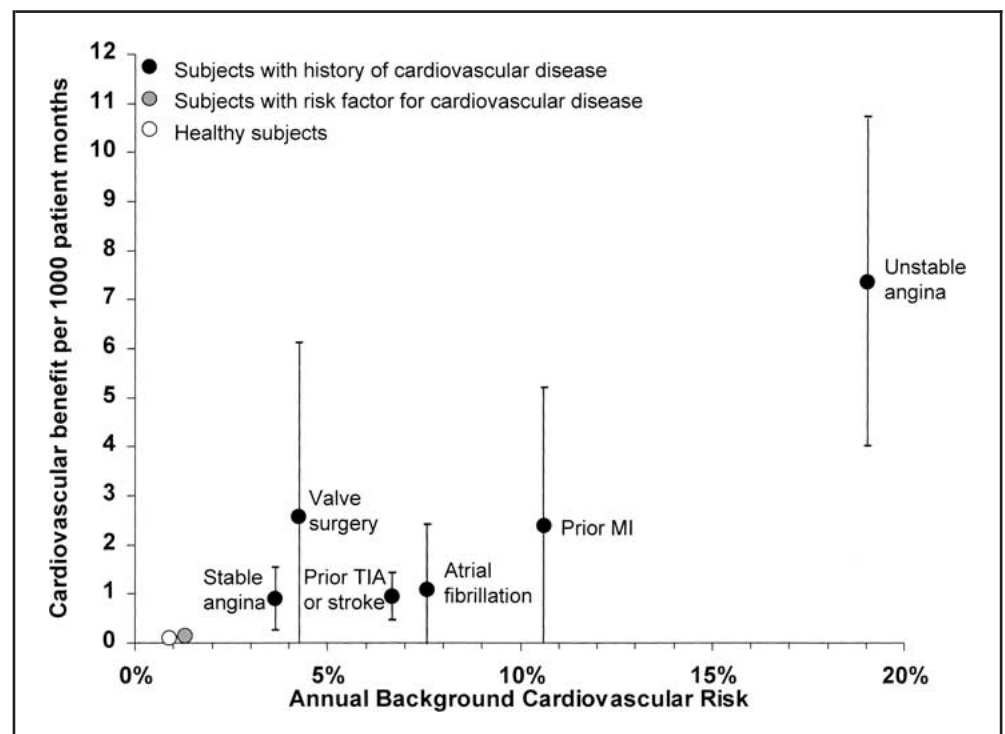
confidence intervals (CIs) are wider than they would be if each point was based on all aspirin trials irrespective of dose. Nevertheless, Fig. 1 illustrates that the cardiovascular benefit of treatment correlates strongly with the background risk across the eight populations (Spearman correlation coefficient = .86). Approximately three additional adverse events are prevented for every 10% increase in annual background risk. The largest potential benefit, not shown in Fig. 1, occurs during the month immediately following an acute MI, wherein 1 month of aspirin treatment prevents approximately 37 vascular events (MI, stroke, or death from all causes) per 1000 persons treated *(50,52)*. Data from patients with acute MI are not included in Fig. 1 because the 1-month benefit cannot be expressed appropriately in annual terms. The cardiovascular benefit of aspirin prophylaxis is smallest in low-risk populations, such as healthy male doctors, in which only 0.09 events are prevented per 1000 patient-months of treatment *(56)*.

The principal adverse effect of aspirin at doses of less than or equal to 500 mg daily is a small increase in the risk of serious bleeding complications. The mean frequency of hemorrhagic complications, per 1000 patient-months of treatment, is 0.07 (95% CI = 0.04 to 0.09). The maximum frequencies are 0.64 and 0.65 in patients undergoing valve surgery and in those with acute MI, respectively. Within each population, the risk of serious bleeding varies with age and comorbidity *(57)*. Serious bleeding complications are sufficiently rare that the risk is offset by potential cardiovascular benefits among patients whose background cardiovascular risk exceeds 3% annually. The risk–benefit balance is equivocal among intermediate-risk patients (i.e., those with a 1%–3% annual incidence of adverse cardiovascular events) and unfavorable among low-risk populations (i.e., those with an annual incidence of <1%). Clinicians should be aware that the calibration of risks and benefits associated with aspirin prophylaxis remains imprecise for any particular patient profile and that prudent treatment decisions require some margin for uncertainty. While aspirin prophylaxis has become standard therapy for patients with diverse high-risk cardiovascular conditions, it has not yet been proven to provide a net clinical benefit in intermediate- or average-risk settings *(52)*.

General mechanism of action of NSAIDs. The mechanism of action that defines NSAIDs as a class is their ability to inhibit the COX activity of the enzyme prostaglandin G/H-synthase and thereby block the biosynthesis of prostaglandins *(58)*. NSAIDs prevent the formation of prostaglandin H_2, the first committed step in the metabolism of arachidonic acid into a complex cascade of signaling lipids, such as prostaglandin D_2, prostaglandin E_2, prostaglandin $F_{2\alpha}$, prostaglandin I_2, and thromboxane, the principal prostanoid metabolite in platelets (Fig. 2). Therapeutic concentrations of NSAIDs (usually in the low micromolar range) are not known to influence other pathways of arachidonic acid metabolism except indirectly by increasing the intracellular concentration of free arachidonic acid, which potentially causes shunting of arachidonic acid through other metabolic pathways (Fig. 2).

Two distinct isoforms of prostaglandin G/H-synthase, designated COX-1 and COX-2, have been recognized since 1991 *(59,60)*. COX-1 is expressed constitutively in many tissues, and it plays a central role in platelet aggregation and gastric cytoprotection *(61,62)*. Although COX-2 is expressed constitutively in the human kidney and brain, its expression is induced in many tissues during inflammation, wound healing, and neoplasia. COX-1 and COX-2 initiate the formation of biologically important prostanoids that coordinate signaling between the cell of origin (autocrine) and neighboring cells (paracrine) by binding to transmembrane G-protein-coupled receptors *(61)*.

NSAIDs vary in their abilities to inhibit COX-1 or COX-2 at different concentrations and in different tissues. For example, aspirin is a relatively selective inhibitor of COX-1 in platelets when given at doses of 50–100 mg daily *(64,65)* but inhibits COX-2 only at plasma concentrations higher than 0.5 m*M*. Most

Fig. 2. Arachidonic acid metabolism. The major metabolites of arachidonic acid produced by the cyclooxygenase (COX) and lipoxygenase (LO) pathways are indicated. Examples of tissues in which individual prostanoids exert prominent effects are indicated **in parentheses.** PGD_2 = prostaglandin D_2; PGE_2 = prostaglandin E_2; $PGF_{2\alpha}$ = prostaglandin $F_{2\alpha}$; TXA_2 = thromboxane A_2; PGI_2 = prostaglandin I_2; HPETE = hydroxyperoxyeicosatetraenoic acid; HETE = hydroxyeicosatetraenoic acid.
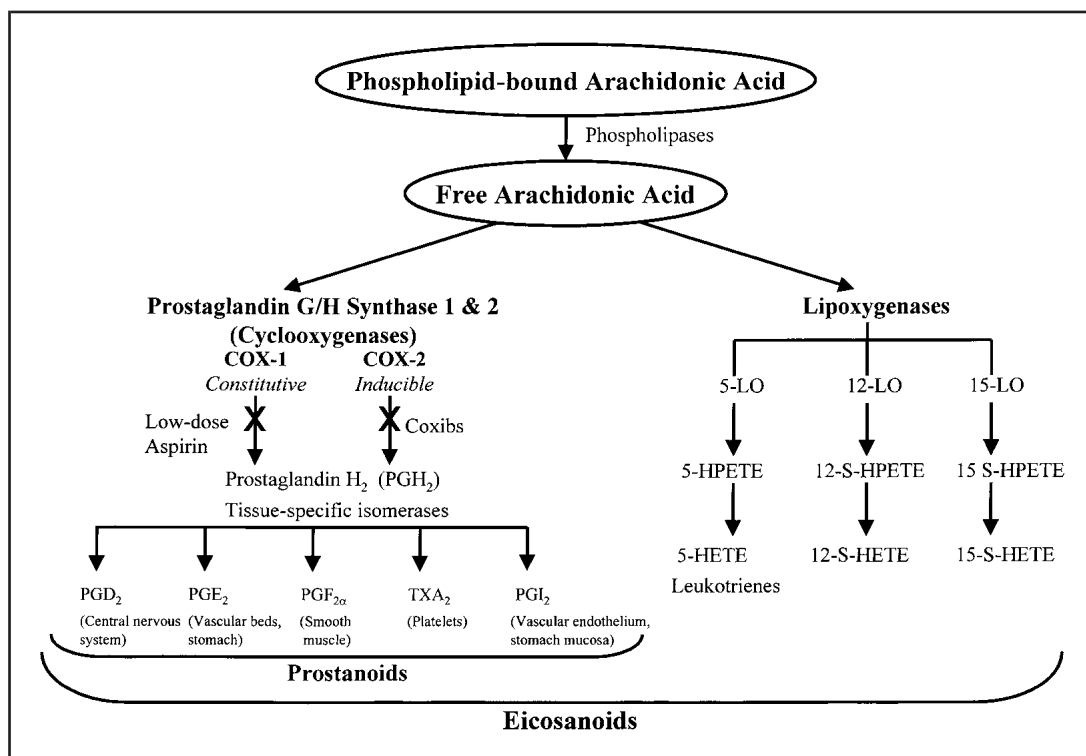
other conventional NSAIDs, such as ibuprofen, sulindac, and indomethacin, inhibit COX-1 and COX-2 to the same extent, whereas a new class of NSAIDs, designated coxibs by the World Health Organization, selectively inhibits COX-2 *(66,67)*. Coxibs were developed to suppress prostanoid formation by COX-2 in inflammation while sparing the protective effects of COX-1 and its products prostaglandin I_2 and prostaglandin E_2 on gastric epithelium. Nevertheless, the availability of coxibs has stimulated research on the role of COX-2 in neoplasia and the potential efficacy and safety of selective COX-2 inhibition against cancer.

The pharmacologic effects of NSAIDs are further complicated by the diverse functions of prostanoids in different tissues and by the variable effects of COX inhibition, depending on drug, dose, and clinical context. The formation of specific prostanoids varies across different tissues because of differences in the concentration of tissue-specific isomerases that catalyze their production from prostaglandin H_2. Furthermore, more than one G-coupled protein receptor may transduce different effects from the same prostanoid *(63)*. The diverse effects of COX inhibition can also be either therapeutic or deleterious, depending on the clinical characteristics of the patient. For example, low-dose aspirin (30–150 mg daily) selectively inhibits the production of thromboxane A_2 in platelets by COX-1, thereby suppressing platelet aggregation, vasoconstriction, and hemostasis *(64,65)*. This antithrombotic effect may be beneficial for the majority of patients at high risk of occlusive vascular disease but deleterious for those who develop bleeding complications. Anti-inflammatory doses of NSAIDs (e.g., ibuprofen at a dose of 800 mg every 8 hours or naproxen at a dose of 500 mg twice daily) are therapeutic for patients with osteoarthritis or rheumatoid arthritis but can cause gastrointestinal ulceration, bleeding, or disruption of renal hemodynamics in susceptible patients. A current controversy concerns whether long-term use of coxibs, which minimize serious gastrointestinal toxicity, may promote thrombosis or offset the cardiovascular benefits of low doses of aspirin by suppressing prostacyclin in vascular endothelial cells *(63)*. Although coxibs do decrease urinary excretion of prostacyclin metabolites in normal subjects *(69–71)*, currently available data do not resolve whether prolonged suppression of COX-2 may adversely affect thrombosis. Given the biologic complexity of prostanoid metabolism, however, it is not surprising that drugs that inhibit the activity of COX isoenzymes can have untoward as well as desirable effects on human health.

Strategies to improve the selectivity of NSAIDs. Several generic strategies have been developed to improve the selectivity and reduce the toxicity of NSAIDs. One strategy is to identify high-risk populations in which the benefits of treatment would outweigh any attendant toxicity. A second strategy, which is taken by pharmaceutical companies, is to develop novel coxibs that inhibit COX-2 and suppress inflammation in chronic arthritis patients while sparing COX-1, thus avoiding the most serious gastrointestinal toxic effects *(67,72)*. Currently available drugs with these properties are celecoxib and rofecoxib. Other highly selective COX-2 inhibitors, such as valdecoxib, etoricoxib, and COX-189, are now completing phase III trials of efficacy and safety in patients with osteoarthritis and rheumatoid arthritis.

A third approach to improve the selectivity and reduce the toxicity of NSAIDs is to determine the lowest effective drug dose and the most critical period for administration to achieve a specific pharmacologic effect. For example, low-dose aspirin (50–100 mg daily) is as effective as aspirin at higher doses in inhibiting COX-1 activity in platelets *(64,65)* and preventing MI and thrombotic stroke *(52)* while minimizing gastrointestinal toxicity. Decisions about the optimal treatment regimen and lowest effective dose are more easily made when the mechanism of action is known. For example, aspirin is the only NSAID that covalently and irreversibly inactivates COX-1, and it does this at concentrations that reach platelets in the enterohepatic circulation but are largely metabolized by the liver before entering the systemic circulation *(53)*. Platelets lack a nucleus and, therefore, cannot synthesize new COX-1 during their 7- to 10-day life span. This combination of factors allows once-daily use of low-dose aspirin to provide full protection against platelet aggregation, despite its half-life of approximately 20 minutes in the systemic circulation *(53)*.

A fourth strategy to improve the balance of benefits and risks associated with NSAID use is to identify combinations of drugs that are effective at very low doses *(73,74)*. For example, much lower doses of sulindac and the cholesterol-lowering drug lovastatin are required to suppress chemically induced cancer in rodents and to stimulate apoptosis in human tumor cells when the drugs are given simultaneously than when either drug is given alone *(75)*. Besides lovastatin, a 3-hydroxy-3-methylglutaryl coenzymeA reductase inhibitor, other drugs that have also been used in combination with either sulindac *(73)*, aspirin *(76)*, or piroxicam *(77,78)* include ornithine decarboxylase inhibitors *(76–78)*, the spice curcumin *(79)*, and EKI-785, an irreversible inhibitor of the epidermal growth factor receptor kinase *(73)*.

Evidence for Cancer Prevention Properties of NSAIDs

Chemically induced intestinal cancer in rodents. The hypothesis that NSAIDs might inhibit the occurrence or growth of colorectal cancer arose in the mid-1970s, when Bennett and Del Tacca *(80)* and Jaffe *(81)* reported that the concentration of prostaglandin E_2 was higher in human colorectal tumor tissue than in the surrounding normal mucosa. These relatively crude measurements of prostaglandin concentrations in human tumors stimulated more than 40 experiments in which numerous NSAIDs were shown to inhibit chemically induced colorectal cancer or aberrant crypt formation in rats or mice *(76–79,82–116)*. In these animal models, weanling rats or mice were given a subcutaneous injection of azoxymethane or other carcinogens known to induce intestinal cancer and were subsequently given known concentrations of NSAIDs in their food or water. Those experiments varied the timing of the NSAID treatment in relation to the carcinogen exposure by initiating treatment before exposure to the carcinogen (initiation phase), during the promotion-progression phase, or both. Colorectal tumors produced in the rat model share many characteristics with human colorectal cancer, except the former have a lower tendency to metastasize *(117)*.

The studies in rodents proved conclusively that aspirin, other conventional NSAIDs (such as piroxicam, indomethecin, sulindac, ibuprofen, and ketoprofen), and selective COX-2 inhibitors [e.g., celecoxib *(107)*] inhibit chemically induced carcinogenesis in rats and mice *(46)*. The highest tolerated dose of nonselective NSAIDs typically reduced the number and size of tumors by 40%–60%. Nonselective NSAIDs suppressed but did not completely eliminate the growth of chemically induced adenomatous polyps and cancers. Two studies of coxibs in this model

(107,114) have indicated that high doses of celecoxib (1500 ppm in food) inhibit 90% of tumors in rats and are better tolerated than comparable doses of nonselective NSAIDs.

At least three important insights can be derived from the rodent experiments. First, nonselective NSAIDs suppress tumor growth to a greater extent and at lower doses when treatment is begun before or coincident with exposure to the carcinogen than when it is delayed until the tumor promotion/progression phase. For example, low-dose piroxicam (25 ppm in food) caused a 30% reduction in tumors when treatment was begun soon after exposure to the carcinogen but only a 12% reduction when treatment was begun 23 weeks after exposure (98). Early initiation of treatment also improves tumor suppression by sulindac sulfone (110) and celecoxib (118). Second, both nonselective and selective NSAIDs effectively inhibit the early stages of tumor development, whereas only selective COX-2 inhibitors are effective when treatment is delayed. For example, celecoxib (1500 ppm in food) reduced tumor incidence and multiplicity by approximately half, even when treatment was delayed until the tumor promotion/progression stage (118). Third, NSAID treatment must be continued without interruption to prevent resumption of tumor growth (91–92).

Experimental studies in rodents have several advantages not offered by other types of studies. First, the ability to administer measured concentrations of single NSAIDs to intact animals provides a greater opportunity to examine complex interactions within and across cells than exists in studies of isolated cell cultures and other *in vitro* models. Second, the rodent experiments are not subject to confounding by lifestyle factors, which may afflict epidemiologic studies. Limitations of the rodent experiments, other than potential interspecies differences, include a lack of uniformity in experimental design that limits comparisons across studies and the lack of measurements of NSAID concentrations in blood, COX-1 activity in platelets, or COX-2 activity in activated monocytes. Measurements of COX-2 activity in activated monocytes could help to identify the enzymatic target involved in tumor inhibition.

Other experimental studies indicate that NSAIDs inhibit many induced and transplanted cancers in various animal models, although the evidence for this is more limited than that for colorectal cancer. Other cancers potentially affected by NSAIDs include tumors of the esophagus (119–121), stomach (122,123), skin (124), breast (125–128), lung (129–132), prostate (133,134), and urinary bladder (135–137). A comprehensive review of these studies is beyond the scope of this review.

Clinical studies and randomized trials of NSAIDs in FAP. Randomized clinical trials have established that two NSAIDs, sulindac (41,42) and celecoxib (44), suppress adenomatous polyps and cause regression of existing polyps in patients with FAP (Table 1). FAP is a rare hereditary condition resulting from germline inactivation of one allele of the adenomatous polyposis coli (APC) gene. Affected individuals develop tens to thousands of adenomatous polyps. If these individuals do not undergo surgical resection of the colon, virtually all develop colorectal cancer by the third or fourth decade of life (138). FAP accounts for only 1% of human colorectal cancers, yet it provides a model of APC inactivation as an early genetic event for the approximately 85% of cancers that develop from sporadic adenomatous polyps. Treatment with both sulindac and celecoxib is used to supplement surgery in FAP patients (139). However, it should be noted that some FAP patients have developed rectal carcinoma, despite ongoing therapy with sulindac (140,141) and that adenomatous polyps resume growth in FAP patients if NSAID prophylaxis is stopped. The one published study that evaluated NSAID prophylaxis in relation to the regression of small (<1 cm) sporadic adenomatous polyps (142) found no statistically significant difference in polyp size among the 18 patients treated with sulindac (300 mg) for 4 months.

Mouse models of FAP. Several murine models that resemble human FAP have been developed and used to determine whether various NSAIDs and coxibs suppress the development of spontaneous intestinal adenomas (143). Nonselective NSAIDs, such as piroxicam (144), sulindac (145–147), and aspirin (148), and selective COX-2 inhibitors, such as celecoxib (144) and rofe-

Table 1. Published randomized clinical trials of NSAIDs and adenomatous colorectal polyps*

Investigator(s), y, (reference No.)	Study population (total No.)	Drug (dose), duration	Phase	Results
Patients with FAP				
Steinbach et al., 2000 (44)	FAP (77)	Celecoxib (100 mg bid or 400 mg bid), 6 mos	II	Celecoxib significantly decreases the No. of colon polyps
Labayle et al., 1991 (41)	FAP (10)	Sulindac (100 mg tid), 4 mos	III	Polyps regressed completely in 6 patients, partly in 3
Giardiello et al., 1993 (42)	FAP (22)	Sulindac (150 mg bid), 9 mos	III	Sulindac decreased No. of polyps by 56% and size by 65%
Nugent et al., 1993 (43)	FAP (24)	Sulindac (400 mg), 6 mos	III	Duodenal polyps <2 mm regressed in 9 of 11 patients treated with sulindac
Other patient populations				
Ladenheim et al. 1995 (142)	Previous adenomatous polyps (44)	Sulindac (300 mg), 4 mos	III	Sulindac did not statistically significantly decrease No. or size of poylps
Ruffin et al., 1997 (203)	High risk of colorectal cancer: FAP, HNPCC (65)	Aspirin (40, 80, 160, 320, or 648 mg qd), 2 wk	I	Lowest effective dose of aspirin to prevent cancer may be 81 mg daily
Carbone et al., 1998 (204)	Dukes' A colon cancer or other conditions (40)	Piroxicam (10 mg qd or 10 mg qod) and DFMO, 6 mos	I	Small doses of DFMO and piroxicam may be additive in their chemopreventive effect
Calaluce et al., 2000 (205)	Previous adenomatous polyps (96)	Piroxicam (7.5 mg), 2 y	IIB	Toxicity of piroxicam treatment may outweigh its benefit
Chow et al., 2000 (206)	Previous adenomatous polyps (27)	Ibuprofen (300 mg qd or 600 mg qd), 4 wk	I	Lowest effective dose of ibuprofen to prevent cancer may be 300 mg daily

*NSAIDs = nonsteroidal anti-inflammatory drugs; FAP = familial adenomatous polyposis; bid = twice a day; tid = three times a day; HNPCC = hereditary nonpolyposis colon cancer; qd = every day; qod = every other day; DFMO = α-difluoromethylornithine.

coxib *(149),* inhibit tumor development in Apc^{Min} mice and other murine models of FAP. These models mimic the rapid development of adenomatous polyps that affects humans with germline inactivation of one APC gene but differ from FAP in that the mouse tumors occur predominantly in the small intestine.

Observational epidemiologic studies in the general population. Numerous (nonrandomized) epidemiologic studies have reported that people who regularly use aspirin and other NSAIDs have a lower incidence of adenomatous polyps and lower incidences of or deaths from colorectal cancer compared with nonusers (Fig. 3) *(4–40).* The consistency of these findings is striking, despite different researchers using various study designs in diverse patient populations. Sustained NSAID use is associated with a 30%–50% reduction in adenomatous polyps, incident disease, and death from colorectal cancer in all but one of these epidemiologic studies *(38),* as seen in Fig. 3, A.

Results from these studies strongly support the hypothesis that NSAIDs inhibit the occurrence and/or progression of colorectal cancer in the general population, not just in FAP patients *(150,151).* The aggregate findings cannot be attributed to earlier detection because of aspirin-induced bleeding or measured potential confounders. Besides attracting substantial research interest and funding to the NSAID hypothesis, the results of these epidemiologic studies suggest that the duration and continuity of NSAID use may be more critical than the daily dose *(12,15,152).* Like the rodent experiments and clinical studies of FAP, the epidemiologic studies also suggest that tumors resume growth after termination of NSAID treatment *(19,30).*

Epidemiologic studies cannot, however, provide randomized evidence that NSAIDs prevent the development of adenomatous polyps or cancer nor have past analyses fully defined the optimal drug, dose, treatment regimen, age to begin prophylactic therapy, or the balance of risks and benefits in different patient populations. Nevertheless, these studies raise an intriguing mechanistic question in that the dose and dosing frequency of aspirin associated with lower risk of colorectal cancer are often insufficient to sustain COX-2 inhibition in nucleated cells *(6,10).* The possibility that activated platelets may contribute to the induction of COX-2 is discussed below.

Other epidemiologic studies have found that prolonged use of NSAIDs is associated with lower incidence of or deaths from cancers at several other sites. The literature regarding these other cancers includes studies of tumors of the esophagus *(7),* stomach *(7),* breast *(5,7,9,16,38,153–157),* lung *(7),* prostate *(7,158),* urinary bladder *(7),* and ovary *(7,159–161).* However, there are fewer studies of these other cancers than of colorectal cancer and the results are less consistent.

Completed randomized trials in the general population. The Physicians' Health Study is the only randomized clinical trial of aspirin in the primary prevention of cardiovascular endpoints of sufficient size to measure incidence or death rates from colorectal cancer, even though the aspirin arm of this trial was terminated after 5 years *(56).* This study showed no reduction in either invasive or *in situ* colorectal cancer incidence nor a reduction in colorectal cancer mortality among 22 071 male physicians who were randomly assigned to receive either 325 mg of aspirin or placebo every other day for 5 years, with a 12-year follow-up *(56).* However, interpretation of these results is limited because of the short duration of randomized treatment, the lack of systematic screening for adenomatous polyps or cancer

at the beginning and end of the trial, and the relatively low dose of aspirin tested.

Mechanistic studies of NSAIDs and apoptosis. Despite continuing uncertainty about the molecular pathways by which NSAIDs may inhibit colorectal neoplasia, there is mounting evidence that tumor inhibition may be mediated by at least two distinct cellular processes. These involve the ability of NSAIDs to restore apoptosis in APC-deficient cells *(162,163)* and their capacity, particularly in the case of coxibs, to inhibit angiogenesis.

Apoptosis, or programmed cell death, is needed to maintain homeostasis in continuously replicating tissues such as the intestine *(164).* Partial suppression of apoptosis occurs early in tumorigenesis in approximately 85% of human colorectal cancers due to the inactivation of both alleles of the APC gene *(165,166).* The suppression of apoptosis allows APC-deficient cells to accumulate in adenomatous polyps. Further suppression of apoptosis occurs as these cells develop additional genetic mutations and phenotypic changes *(167).*

In vitro, both nonselective NSAIDs and selective COX-2 inhibitors stimulate apoptosis in APC-deficient cells that have not yet undergone malignant transformation. This is also seen clinically in FAP patients treated with sulindac *(168)* and in experimental studies of Apc^{Min} mice *(145,147,148,169)* and rats exposed to chemical carcinogens *(170).* Nonselective NSAIDs lose their ability to inhibit chemically induced tumors when polyps undergo malignant transformation. In contrast, selective COX-2 inhibitors stimulate apoptosis and suppress growth in many carcinomas, including cultured human cancers of the stomach *(171),* esophagus *(121,172),* tongue *(173),* brain *(174),* lung *(130),* and pancreas *(175).*

The precise mechanism by which NSAIDs restore apoptosis remains controversial *(176),* although it clearly affects factors related to APC deficiency or the induction of COX-2 or both. Apoptosis can be suppressed in normal human or rodent intestinal epithelial cells by manipulating these cells to overexpress COX-2 *(162,177).* In human HT-29 colon cancer cells, apoptosis can be restored by treatment with selective *(178)* or nonselective *(179,180)* COX inhibitors or by restoring APC gene function *(165).* Apoptosis becomes progressively more inhibited during the development of colorectal cancer *(167),* coincident with the increasing expression of COX-2. For example, COX-2 is undetectable in normal epithelium but is detectable in 40% of adenomatous polyps *(181)* and in more than 80% of colorectal cancers. COX-2 expression in human colorectal carcinomas is associated with larger tumor size and deeper invasion, although not with metastases *(182).*

Other studies *(183,184)* suggest that COX-1 activity, perhaps through the induction of COX-2, may also be essential for the development of colorectal neoplasia. In mouse knockout studies *(183,184),* deletion of either the COX-1 or COX-2 genes in Apc-deficient mice caused a 70%–80% reduction in intestinal polyposis. We have hypothesized that COX-1 activity in activated platelets may signal the increased expression of COX-2 in other cells through the release of lipid or protein paracrine mediators *(185).* A role for COX-1 in the induction of COX-2 might explain why, in epidemiologic studies, aspirin use is associated with reduced risk of colorectal cancer even at doses and dosing intervals that could not sustain COX-2 inhibition in nucleated cells *(6,12).*

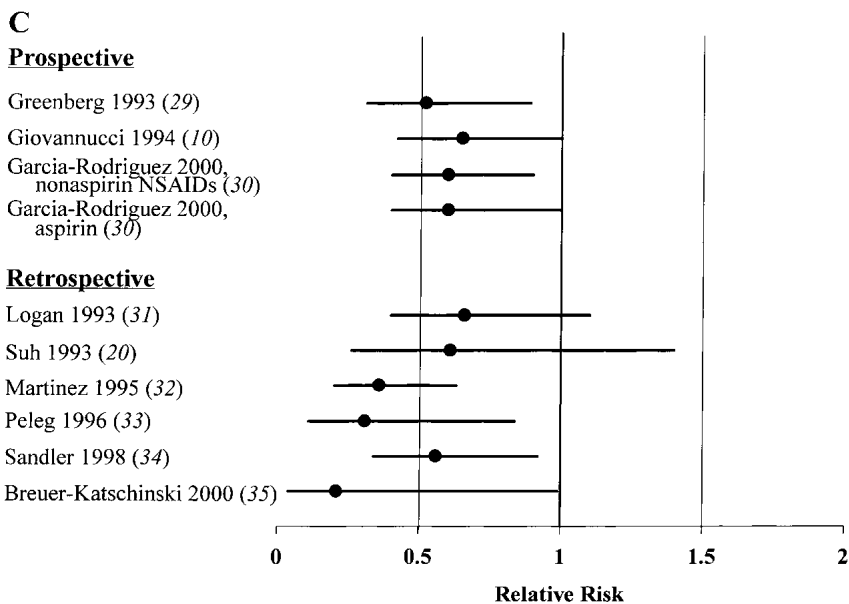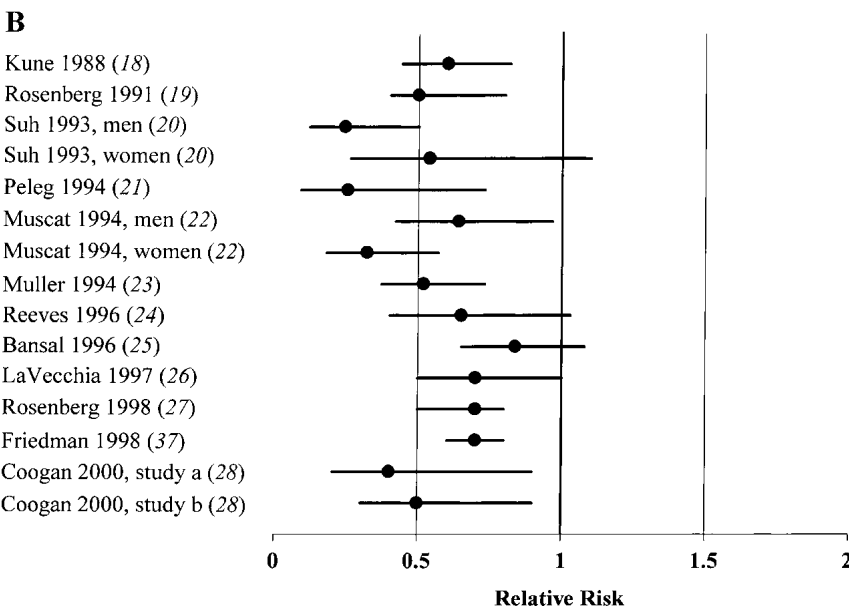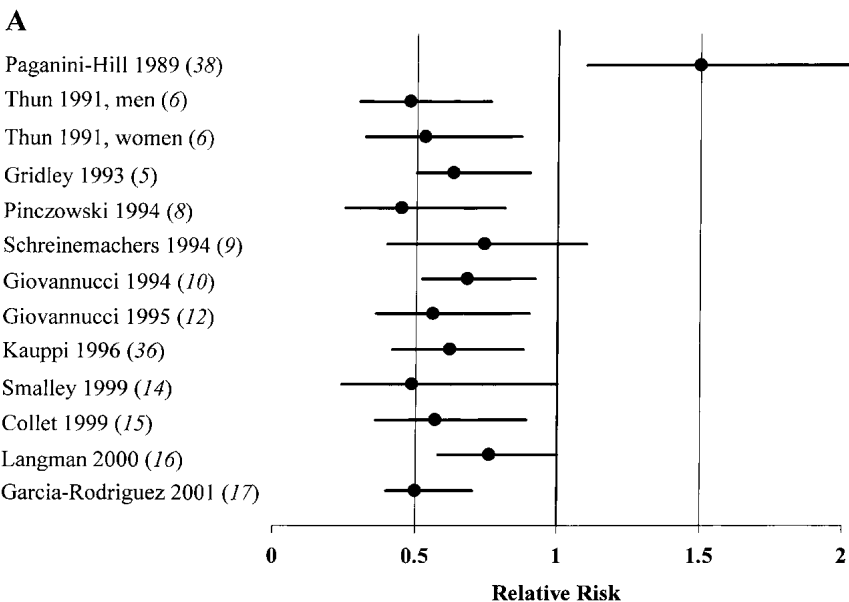Despite these observations, results from other studies chal-

A

Paganini-Hill 1989 (*38*)
Thun 1991, men (*6*)
Thun 1991, women (*6*)
Gridley 1993 (*5*)
Pinczowski 1994 (*8*)
Schreinemachers 1994 (*9*)
Giovannucci 1994 (*10*)
Giovannucci 1995 (*12*)
Kauppi 1996 (*36*)
Smalley 1999 (*14*)
Collet 1999 (*15*)
Langman 2000 (*16*)
Garcia-Rodriguez 2001 (*17*)

Relative Risk

B

Kune 1988 (*18*)
Rosenberg 1991 (*19*)
Suh 1993, men (*20*)
Suh 1993, women (*20*)
Peleg 1994 (*21*)
Muscat 1994, men (*22*)
Muscat 1994, women (*22*)
Muller 1994 (*23*)
Reeves 1996 (*24*)
Bansal 1996 (*25*)
LaVecchia 1997 (*26*)
Rosenberg 1998 (*27*)
Friedman 1998 (*37*)
Coogan 2000, study a (*28*)
Coogan 2000, study b (*28*)

Relative Risk

C

Prospective

Greenberg 1993 (*29*)
Giovannucci 1994 (*10*)
Garcia-Rodriguez 2000, nonaspirin NSAIDs (*30*)
Garcia-Rodriguez 2000, aspirin (*30*)

Retrospective

Logan 1993 (*31*)
Suh 1993 (*20*)
Martinez 1995 (*32*)
Peleg 1996 (*33*)
Sandler 1998 (*34*)
Breuer-Katschinski 2000 (*35*)

Relative Risk

Fig. 3. Epidemiologic studies of the association between nonsteroidal anti-inflammatory drug (NSAID) use and colorectal cancer or adenomatous polyps. The relative risk estimates (**circles**) and 95% confidence intervals (**lines**) refer to the incidence or death rate among regular NSAID users compared with that among nonusers in **A**) cohort studies, **B**) case–control studies of NSAIDs and colorectal cancer, and **C**) studies of NSAIDs and adenomatous polyps.

lenge the conventional wisdom that COX inhibition is the only shared function of NSAIDs *(186)* or that the products rather than the substrate of COX activity mediate its biologic effects. For example, in some experimental models, the concentration of free arachidonic acid itself regulates apoptosis in colorectal epithelial cells *(187,188)*. Other experimental models suggest that NSAIDs may affect apoptosis through a mixture of prostaglandin-dependent and prostaglandin-independent pathways *(176)*. The selective COX-2 inhibitor NS-398 stimulates apoptosis in human S/KS colon carcinoma cells, which do not express COX-2 enzyme, as well as in HT-29 cells, which do *(178)*. Although sulindac sulfone is believed not to inhibit COX activity, it nonetheless stimulates apoptosis in rats exposed to chemical carcinogens *(110)* and in human HT-29 colon carcinoma cells *(189)*. High concentrations of sulindac sulfone and sodium salicylate reportedly modify signal transduction through either the c-MYC oncogene *(190)*, nuclear factor-κB *(191,192)*, or p38, a mitogen-activated protein kinase *(193,194)*. Very high concentrations of sulindac sulfide inhibit transcriptional activation by the nuclear peroxisome proliferator-activated receptor-δ *(163)*, a nuclear hormone receptor regulated partly by APC gene function *(163,195)*. NSAIDs have also been reported to induce apoptosis through 15-lipoxygenase-1, independent of COX-2 *(196)*. However, many of these effects have been demonstrated only with high concentrations of NSAIDs *in vitro* and are of uncertain clinical relevance.

Mechanistic studies of angiogenesis. A second cellular process by which COX-2 inhibitors may inhibit tumor growth is through inhibition of angiogenesis and neovascularization *(197,198)*. Solid tumors must stimulate the formation of new capillary blood vessels to grow larger than approximately 2 mm in diameter *(198–200)*. COX-2 expression is widely induced in the angiogenic vasculature of colorectal adenomatous polyps and in carcinomas of the colon, lung, breast, esophagus, and prostate *(200,201)*. Selective COX-2 inhibitors suppress the growth of corneal capillary blood vessels in rats exposed to basic fibroblast growth factor *(201)* and inhibit the growth of several human tumors transplanted into mice *(201,202)*. Therapeutic (low micromolar) concentrations of coxibs also suppress the release of angiogenic growth factors by human or rodent colorectal cancer cells that are cocultured with vascular endothelial cells *(197,198)* and block migration and tube formation by the endothelial cells. In contrast, toxic concentrations of aspirin *(197)* or indomethacin *(198)* are required to block vascular endothelial tube formation. These experiments suggest that COX-2 may be essential for tumor vascularization and growth. However, the relevance of the experimental models to human colorectal cancer remains uncertain.

Implications for Ongoing and Future Scientific Research

Randomized trials of NSAIDs and prevention of colorectal cancer. Approximately 20 randomized clinical trials designed to test whether nonselective NSAIDs or coxibs inhibit the early development of colorectal cancer either have been completed (Table 1) or are ongoing (Table 2). Most are phase I and II studies to determine the bioactivity of various NSAID treatments in rectal epithelium and/or their gastric toxicity *(203–206)*. We believe that the ongoing phase I studies could be strengthened by testing validated biomarkers of apoptosis and angiogenesis in adenomatous polyps and early-stage carcinomas. For example, quantitative measures of apoptosis in adeno-

matous polyps may be a more sensitive index of the biologic effect of NSAIDs than are measures of cell proliferation, aberrant crypt formation, or prostaglandin concentrations in normal rectal mucosa. Improved biomarkers are essential for phase I/II studies to identify the least toxic treatments and drug combinations for further testing.

Seven phase III trials are currently testing whether aspirin, other nonselective NSAIDs, or selective COX-2 inhibitors suppress the development of adenomatous polyps or cancer in high-risk patients (Table 2). These prevention trials will provide the first randomized evidence of the effectiveness of aspirin at doses of 80–300 mg daily or celecoxib at doses of 200–400 mg daily in inhibiting the development of sporadic adenomatous polyps in patients without FAP. Two of the studies are also testing whether aspirin and celecoxib inhibit colorectal cancer among patients with HNPCC, a condition that accounts for approximately 15% of colorectal cancers.

Measuring surrogate endpoints in high-risk populations improves the feasibility of cancer prevention studies, but it also introduces new sources of uncertainty. The prevention of adenomatous polyps is not synonymous with the prevention of colorectal cancer, nor are findings in patients with FAP or HNPCC necessarily generalizable to patients with most colorectal cancers. Furthermore, even if NSAIDs do reduce the risk of colorectal cancer, none of these trials will address the safety of administering prolonged treatment prophylactically to healthy people. However, the evaluation of appropriately validated biomarkers *(164)* could strengthen the ability of phase III trials to test focused mechanistic hypotheses by verifying that the intervention being tested achieves the desired pharmacologic effect under field conditions.

Trials of NSAIDs as adjuvant therapy. New trials are testing the efficacy of coxibs to treat precancerous lesions of the mouth, esophagus, and skin and as adjuvant therapy for solid tumors that express COX-2 (Table 3) *(172,175,207–211)*. Therapeutic trials are easier to conduct and are more cost-effective than prevention trials because of their smaller size and shorter follow-up *(201)*. Therapeutic trials can directly measure clinical endpoints, such as tumor recurrence and survival, as well as potential surrogate measures of disease. Furthermore, the high risk of cancer recurrence or progression in therapeutic settings offsets some of the constraints on toxicity that limit the prevention trials.

A strong scientific rationale supports therapeutic trials of coxibs for the treatment of cancers that express COX-2 in vascular endothelium *(172,175,207–211)*. First, celecoxib suppresses the growth of human colon and lung tumors xenografted into rodents *(212)*. Second, adjuvant treatment with coxibs enhances the response of human HT-29 colon cancer cells and mouse sarcoma transplants to standard chemotherapy or radiation therapy *(213,214)*. Third, a cross-sectional clinical study has shown that COX-2 expression in human colorectal tumors is directly associated with tumor stage and size at diagnosis and is inversely associated with patient survival *(215)*. Evidence from randomized trials suggesting that coxibs inhibit recurrence or prolong survival in early-stage colorectal cancer would stimulate further trials of adjuvant treatment of other COX-2-expressing solid tumors.

Future roles for epidemiologic studies. An important continuing role for epidemiologic studies is to quantify the benefits and risks of NSAID treatment across a broader range of treat-

Table 2. Ongoing clinical trials of NSAIDs and colorectal adenomatous polyps*

Principal investigator: location (protocol No.)	Endpoint	Study population (total No.)	Drug (dose),† duration	Phase	Years
Aspirin study					
Sinicrope: M.D. Anderson-CAPT Study (DM93-129)‡	Adenomatous polyps	Previous adenomatous polyps (112)	Aspirin (81 mg qd), 12 wk	II	2000–2001
Schilsky: U Chicago/Sandler: UNC (NCI-P93-0048)§	Adenomatous polyps, survival	Early/late-stage Dukes' A and B (900)	Aspirin (325 mg qd), 4 y	III	1993–2003
Baron: Dartmouth (NCI-P95-0063)§	Adenomatous polyps	Previous adenomatous polyps (700)	Aspirin (80 mg qd or 325 mg qd) and/or folate, 3 y	III	1994–2001
Logan: Nottingham, UK‖	Adenomatous polyps	Previous adenomatous polyps (1000)	Aspirin (300 mg qd) and/or folic acid (500 μg qd), 3 y	III	1997–2003
Burn: Newcastle-upon-Tyne, UK CAPP1-FAP study‖	Adenomatous polyps	FAP carriers (400)	Aspirin (600 mg qd) and/or resistant starch (30 g), 1 y	III	1993–na
Burn: Newcastle-upon-Tyne, UK CAPP2-HNPCC study‖	Adenomatous polyps	HNPCC carriers (1000)	Aspirin (600 mg qd) and/or resistant starch (30 g), 2 y	III	1999–2001
Other nonselective NSAIDS study					
Shiff: Rockefeller (NCI-V98-1425)§	Effects on colonic epithelium and mucosa, dosage	Average or above average risk of colon cancer (130)	Sulindac and curcumin (dose na), 6–10 wk	na	1996–na
Holt: Columbia (NCI-P97-0110)§	Effects on colon crypt and rectal epithelium	Previous adenomatous polyps (40)	Sulindac (150 mg bid), 6 mos	II	1995–2000
Meyskens: UC Irvine (NCI-P00-0150)§	Adenomatous polyps	Previous adenomatous polyps, no FAP (240)	Sulindac (150 mg qd) and DFMO (500 mg/m² qd), 3 y	IIB	1997–2001
Selective COX-2 inhibitors study					
Kirsch: NCI (NCI-98-C-0087)§	Bioactivity (COX-2 and PGE2 expression), tolerability, safety	HNPCC patients and gene carriers (81)	Celecoxib, 1 y	I/II	1998–2002
Bertagnolli: Brigham and Women's Hospital (NCI-P00-0141)§	Adenomatous polyps	Previous sporadic adenomatous polyps (1000)	Celecoxib, 3 y	III	2000–na
Alberts: Arizona Cancer Center¶	Adenomatous polyps	Previous sporadic adenomatous polyps (1600)	Celecoxib alone and with selenium, 3–5 y	III	2001–2006
Mortensen: Merck#	Adenomatous polyps	Previous sporadic adenomatous polyps (2400)	Rofecoxib, 3 y	III	2001–2004

*NSAIDs = nonsteroidal anti-inflammatory drugs; PI = principal investigator; CAPT = Calcium Aspirin Prevention Trial; qd = every day; UNC = University of North Carolina; NCI = National Cancer Institute; UK = United Kingdom; CAPP1 = Concerted Action Polyposis Prevention 1; FAP = familial adenomatous polyposis; HNPCC = hereditary nonpolyposis colon cancer; CAPP2 = Concerted Action Polyposis Prevention 2; na = not available; bid = twice a day; UC = University of California; DFMO = α-difluoromethylornithine; PGE2 = prostaglandin E_2.

†Information on endpoint, dose, and end of study not available for some studies.

‡Clinical Trials, The University of Texas M. D. Anderson Cancer Center (http://www.mdanderson.org/patients_public/clinical_trials/).

§PDQ® Clinical Trials Database, National Cancer Institute (http://cancernet.nci.nih.gov/trialsrch.shtml).

‖Current Controlled Trials, Medical Research Council (http://www.controlled-trials.com/)

¶Press Release, Arizona Cancer Center (http://www.azcc.arizona.edu/in_print/p_release/p_colon-cancer-grant.htm.

#Press Release, University of Pittsburgh Cancer Institute (http://www.upci.upmc.edu/internet/news/upci_news/2001/040201_colon_vioxx_study.html).

ment regimens, outcomes, and patient populations than is feasible in randomized clinical trials. Whereas epidemiologic studies can assess multiple endpoints and prolonged exposures, randomized studies must generally be stopped when benefit or toxicity is demonstrated conclusively. Epidemiologic studies can assess how cofactors, such as age and comorbidity, affect the likelihood of benefits and risks in different patient populations. Ultimately, clinical guidelines should enable a practicing physician to consider how NSAIDs affect multiple health endpoints (cancer, cardiovascular, and other) in specific patient populations.

Basic research. Studies of chemically induced colorectal cancer in rats have established that numerous NSAIDs inhibit colorectal neoplasia in this model. Studies of whole animals will continue to be important for identifying the cellular targets and molecular pathways by which this inhibition occurs. Epithelial cells do not exist in a vacuum. Research on whole animals reveals the complex interactions that occur between adjoining tissues or between epithelial cells and the underlying stroma *(216).* The development of drugs that are more selective and

effective than those currently available will require a better understanding of the complex intracellular signaling that occurs in intact animals but may not be replicated by cell culture or other laboratory models *(217,218).*

Studies using transgenic animals can also identify the genetic and epigenetic effects of NSAIDs. Deletion of the genes that code for enzymes (e.g., inducible prostaglandin E_2 synthase) and receptors (e.g., the thromboxane receptor) downstream of prostaglandin G/H synthase may help to characterize the lipid mediator(s) involved in the modulation of apoptosis and angiogenesis. Additional mechanistic insights can be obtained by measuring NSAID concentrations in plasma, COX-1 activity in circulating platelets, and COX-2 activity in activated monocytes.

CONCLUSIONS

Numerous experimental, clinical, and epidemiologic studies indicate that NSAIDs, particularly the highly selective COX-2 inhibitors, show promise as anticancer drugs. The clinical application of these drugs is still limited by the lack of randomized

Table 3. Therapeutic trials of selective COX-2 inhibitors and endpoints other than colorectal adenomatous polyps or cancer*

Principal Investigator: location (protocol No.)	Endpoint	Study population (Total No.)	Drug (dose),† duration	Phase	Years
Selective COX-2 inhibitors study					
Mulshine: NCI (NCI-98-C-0118)‡	Efficacy (leukoplakia reversal, histological changes,) biomarkers, safety	Oropharyngeal leukoplakia (57)	Ketorolac rinse, 3 mos	IIB	1998–2001
Boyle: Memorial Sloan-Kettering (NCI-G01-1930)	Efficacy (clinical & histological response), safety	Oral premalignant lesions (84)	Celecoxib, 12 wk	II	2000–2001
Forastiere: Johns Hopkins (NCI-P00-0145)‡	Dysplasia regression	Low or high grade Barrett's esophageal dysplasia (200)	Celecoxib, 48–96 wk	II	2000–na
Dawsey: NCI (Linxian, China) (NCI-OH95-C-N026)‡	Dysplasia regression	Esophageal squamous dysplasia (240-600)	Celecoxib and selenium, 1 y	II	1999–2000
Elmets: UAB (NCI-P00-0161)‡	Regression/prevention of actinic keratoses, biomarkers, safety	Actinic keratoses (300)	Celecoxib, 1 y	II/III	2000–na
Sabichi: M.D. Anderson (NCI-P00-0165)‡	Time to recurrence, biomarkers, toxicity	Superficial transitional cell bladder carcinoma (200)	Celecoxib, 1–2 y	II/III	2000–na
Dang: Memorial Sloan-Kettering (NCI-G00-1869)‡	Efficacy, safety	Metastatic breast cancer (12–25)	Celecoxib and trastuzumab, duration na	II	2000–na
Carducci: Johns Hopkins (NCI-P01-0186)‡	Biomarker (prostaglandin levels), toxicity	Localized prostate cancer (60–70)	Celecoxib, 6 wk	I	2001–na

*PI = principal investigator; NCI = National Cancer Institute; na = not available; UAB = University of Alabama, Birmingham.

evidence of their efficacy in populations other than those with FAP and against endpoints other than adenomatous colorectal polyps. In addition, unresolved questions about the mechanism(s) by which these drugs act, the optimal drug, dose, treatment regimen, and the balance of risks and benefits in specific populations must be answered. We hope that the issues raised by this review will help to sustain progress in this exciting area.

REFERENCES

(1) Taketo M. Cyclooxygenase-2 inhibitors in tumorigenesis (part I). J Natl Cancer Inst 1998;90:1529–36.

(2) Taketo M. Cyclooxygenase-2 inhibitors in tumorigenesis (part II). J Natl Cancer Inst 1998;90:1609–20.

(3) Janne PA, Mayer RJ. Chemoprevention of colorectal cancer. N Engl J Med 2000;342:1960–8.

(4) Isomaki HA, Hakulinen T, Joutsenlahti U. Excess risk of lymphomas, leukemia and myeloma in patients with rheumatoid arthritis. J Chronic Dis 1978;31:691–6.

(5) Gridley G, McLaughlin JK, Ekbom A, Klareskog L, Adami HO, Hacker DG, et al. Incidence of cancer among patients with rheumatoid arthritis. J Natl Cancer Inst 1993;85:307–11.

(6) Thun MJ, Namboodiri MM, Heath CJ Jr. Aspirin use and reduced risk of fatal colon cancer. N Engl J Med 1991:325:1593–6.

(7) Thun MJ, Namboodiri MM, Calle EE, Flanders WD, Heath CW Jr. Aspirin use and risk of fatal cancer. Cancer Res 1993;53:1322–7.

(8) Pinczowski D, Ekbom A, Baron J, Yuen J, Adami HO. Risk factors for colorectal cancer in patients with ulcerative colitis: a case–control study. Gastroenterology 1994;107:117–20.

(9) Schreinemachers DM, Everson RB. Aspirin use and lung, colon, and breast cancer incidence in a prospective study. Epidemiology 1994;5:138–46.

(10) Giovannucci E, Rimm EB, Stampfer M, Colditz G, Asherio A, Willett W. Aspirin use and the risk for colorectal cancer and adenoma in male health professionals. Ann Intern Med 1994;121:241–6.

(11) Giovannucci E, Willett W. Dietary factors and risk of colon cancer. Ann Intern Med 1994;26:443–52.

(12) Giovannucci E, Egan KM, Hunter DJ, Stampfer MJ, Colditz GA, Willett WC, et al. Aspirin and the risk of colorectal cancer in women. N Engl J Med 1995;333:609–14.

(13) Giovannucci E, Rimm EB, Ascherio A, Stampfer MJ, Colditz GA, Willett WC. Alcohol, low-methionine–low-folate diets, and risk of colon cancer in men. J Natl Cancer Inst 1995;87:265–73.

(14) Smalley W, Ray WA, Daugherty J, Griffin MR. Use of nonsteroidal anti-inflammatory drugs and incidence of colorectal cancer. Arch Intern Med 1999;159:161–6.

(15) Collet JP, Sharpe C, Belzile E, Boivin JF, Hanley J, Abenhaim L. Colorectal cancer prevention by non-steroidal anti-inflammatory drugs: effects of dosage and timing. Br J Cancer 1999;81:62–8.

(16) Langman MJ, Cheng KK, Gilman EA, Lancashire RJ. Effect of anti-inflammatory drugs on overall risk of common cancer: case–control study in general practice research database. BMJ 2000;320:1642–6.

(17) Garcia-Rodriguez LA, Huerta-Alvarez C. Reduced risk of colorectal cancer among long-term users of aspirin and nonaspirin nonsteroidal antiinflammatory drugs. Epidemiology 2001;12:88–93.

(18) Kune G, Kune S, Watson LF. Colorectal cancer risk, chronic illnesses, operations, and medications: case control results from the Melbourne Colorectal Cancer Study. Cancer Res 1988;48:4399–404.

(19) Rosenberg L, Palmer JR, Zauber AG, Warshauer ME, Stolley PP, Shapiro S. A hypothesis: nonsteroidal anti-inflammatory drugs reduce the incidence of large-bowel cancer. J Natl Cancer Inst 1991;83:355–8.

(20) Suh O, Mettlin C, Petrelli N. Aspirin use, cancer, and polyps of the large bowel. Cancer 1993;72:1171–7.

(21) Peleg I, Maibach H, Brown SH, Wilcox CM. Aspirin and nonsteroidal anti-inflammatory drug use and the risk of subsequent colorectal cancer. Arch Intern Med 1994;154:394–9.

(22) Muscat J, Stellman SD, Wynder EL. Nonsteroidal antiinflammatory drugs and colorectal cancer. Cancer 1994;74:1847–54.

(23) Muller A, Sonnenberg A, Wasserman IH. Diseases preceding colon cancer: a case–control study among veterans. Dig Dis Sci 1994;39:2480–4.

(24) Reeves MJ, Newcomb PA, Trentham-Diez A, Storer BE, Remington P. Nonsteroidal anti-inflammatory drug use and protection against colorectal cancer in women. Cancer Epidemiol Biomarkers Prev 1996;5:955–60.

(25) Bansal P, Sonnenberg A. Risk factors of colorectal cancer in inflammatory bowel disease. Am J Gastroenterol 1996;91:44–8.

(26) LaVecchia C, Negri E, Francheschi S, Conti E, Montella M, Giacosa A, et al. Aspirin and colorectal cancer. Br J Cancer 1997;76:675–7.

(27) Rosenberg L, Louik C, Shapiro S. Nonsteroidal antiinflammatory drug use and reduced risk of large bowel carcinoma. Cancer 1998;82:2326–33.

(28) Coogan PF, Rosenberg L, Louik C, Zauber AG, Stolley PD, Strom BL, et al. NSAIDs and risk of colorectal cancer according to presence or absence of family history of the disease. Cancer Causes Control 2000;11:249–55.

(29) Greenberg ER, Baron JA, Freeman DH Jr, Mandel JS, Haile R. Reduced risk of large-bowel adenomas among aspirin users. The Polyp Prevention Study Group. J Natl Cancer Inst 1993;85:912–6.

(30) Garcia-Rodriguez LA, Huerta-Alvarez C. Reduced incidence of colorectal adenoma among long-term users of nonsteroidal antiinflammatory drugs: a pooled analysis of published studies and a new population-based study. Epidemiology 2000;11:376–81.

(31) Logan RF, Little J, Hawtin PG, Hardcastle JD. Effect of aspirin and non-steroidal anti-inflammatory drugs on colorectal adenomas: case–control study of subjects participating in the Nottingham faecal occult blood screening programme. BMJ 1993;307:285–9.

(32) Martinez M, McPherson RS, Levin B, Annegers JF. Aspirin and other nonsteroidal anti-inflammatory drugs and risk of colorectal adenomatous polyps among endoscoped individuals. Cancer Epidemiol Biomarkers Prev 1995;4:703–7.

(33) Peleg II, Lubin MF, Cotsonis GA, Clark WS, Wilcox CM. Long-term use of nonsteroidal antiinflammatory drugs and other chemopreventors and risk of subsequent colorectal neoplasia. Dig Dis Sci 1996;41:1319–26.

(34) Sandler RS, Galanko JC, Murray SC, Helm JF, Woosley JT. Aspirin and nonsteroidal anti-inflammatory agents and risk for colorectal adenomas. Gastroenterology 1998;114:441–7.

(35) Breuer-Katschinski B, Nemes K, Rump B, Leiendecker B, Marr A, Breuer N, et al. Long-term use of nonsteroidal antiinflammatory drugs and the risk of colorectal adenomas. The Colorectal Adenoma Study Group. Digestion 2000;61:129–34.

(36) Kauppi M, Pukkala E, Isomaki H. Low incidence of colorectal cancer in patients with rheumatoid arthritis. Clin Exp Rheumatol 1996;14:551–3.

(37) Friedman GD, Coates AO, Potter JD, Slattery ML. Drugs and colon cancer. Pharmacoepidemiol Drug Safety 1998;7:99–106.

(38) Paganini-Hill A, Chao A, Ross R, Henderson B. Aspirin use and chronic diseases: a cohort study of the elderly. Br Med J 1989;299:1247–50.

(39) Paganini-Hill A, Hsu G, Ross RK, Henderson BE. Aspirin use and incidence of large-bowel cancer in a California retirement community [letter]. J Natl Cancer Inst 1991;83:1182–3.

(40) Paganini-Hill A. Aspirin and colorectal cancer: the Leisure World cohort revisited. Prev Med 1995;24:113–5.

(41) Labayle D, Fischer D, Vielh P, Drouhin F, Pariente A, Bories C, et al. Sulindac causes regression of rectal polyps in familial adenomatous polyposis. Gastroenterology 1991;101:635–9.

(42) Giardiello FM, Hamilton SR, Krush AJ, Piantadosi S, Hylind LM, Celano P, et al. Treatment of colonic and rectal adenomas with sulindac in familial adenomatous polyposis. N Engl J Med 1993;328:1313–6.

(43) Nugent KP, Farmer KC, Spigelman AD, Williams CB, Phillips RK. Randomized controlled trial of the effect of sulindac on duodenal and rectal polyposis and cell proliferation in patients with familial adenomatous polyposis. Br J Surg 1993;80:1618–9.

(44) Steinbach G, Lynch PM, Phillips RK, Wallace MH, Hawk E, Gordon GB, et al. The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis. N Engl J Med 2000;342:1946–52.

(45) Marcus A. Aspirin as prophylaxis against colorectal cancer. N Engl J Med 1995;333:656–8.

(46) International Agency for Research on Cancer (IARC). Non-steroidal anti-inflammatory drugs. In: IARC Handbooks of Cancer Prevention. Lyon (France): IARC Press; 1997.

(47) Howe HL, Wingo PA, Thun MJ, Ries LA, Rosenberg HM, Feigal EG, et al. Annual report to the nation on the status of cancer (1973 through 1998), featuring cancers with recent increasing trends. J Natl Cancer Inst 2001;93:824–42.

(48) Greenlee RT, Hill-Harmon MB, Murray T, Thun M. Cancer statistics, 2001. CA Cancer J Clin 2001;51:15–36.

(49) American Society of Clinical Oncology. Hereditary colorectal cancer syndromes. In: Cancer genetics & cancer predisposition testing. Vol. 1. Alexandria (VA): American Society of Clinical Oncology; 1998.

(50) Antiplatelet Trialists' Collaboration. Collaborative overview of randomised trials of antiplatelet therapy—I: prevention of death, myocardial infarction, and stroke by prolonged antiplatelet therapy in various categories of patients. BMJ 1994;308:81–106.

(51) Antithrombotic Trialists' Collaboration. Collaborative meta-analysis of randomised trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients. BMJ. 2002;324:71–86.

(52) Patrono C, Coller B, Dalen JE, FitzGerald GA, Fuster V, Gent M, et al. Platelet-active drugs: the relationships among dose, effectiveness, and side effects. Chest 2001;119(1 Suppl):39S–63S.

(53) Patrono C. Aspirin as an antiplatelet drug. N Engl J Med 1994;330:1287–94.

(54) Hansson L, Zanchetti A, Carruthers SG, Dahlof B, Elmfeldt D, Julius S, et al. Effects of intensive blood pressure lowering and low-dose aspirin in patients with hypertension: principal results of the Hypertension Optimal Treatment (HOT) randomised trial. Lancet 1998;351:1755–62.

(55) Thrombosis prevention trial: randomised trial of low-intensity oral anticoagulation with warfarin and low-dose aspirin in the primary prevention of ischaemic heart disease in men at increased risk. The Medical Research Council's Practice Research Framework. Lancet 1998;351:233–41.

(56) Steering Committee of the Physicians' Health Study Research Group. Final report on the aspirin component of the ongoing Physicians' Health Study. N Engl J Med 1989;321:129–35.

(57) Wolfe MM, Lichtenstein DR, Singh G. Gastrointestinal toxicity of nonsteroidal antiinflammatory drugs. N Engl J Med 1999;340:1888–99.

(58) Vane JR, Flower RJ, Botting RM. History of aspirin and its mechanism of action. Stroke 1990;21(12 Suppl 4):12–23.

(59) Smith WL, Garavito RM, DeWitt D. Prostaglandin endoperoxide H synthases (cyclooxygenases)-1 and -2. J Biol Chem 1996;271:33157–60.

(60) Xie W, Robertson D, Simmons D. Mitogen-inducible prostaglandin G/H synthase: a new target for nonsteroidal antiinflammatory drugs. Drug Dev Res 1992;25:249–65.

(61) Morrow JD, Roberts LJ. Lipid-derived autacoids. In: Hardman JG, Limbird LE, editors. The pharmacological basis of therapeutics. New York (NY): McGraw-Hill; 2001. p. 669–85.

(62) Roberts LJ, Morrow JD. Analgesic-antipyretic and antiinflammatory agents and drugs employed in the treatment of gout. In: Hardman JG, Limbird LE, editors. The pharmacological basis of therapeutics. New York (NY): McGraw-Hill; 2001. p. 687–731.

(63) FitzGerald GA, Patrono C. The coxibs, selective inhibitors of cyclooxygenase-2. N Engl J Med 2001;345:433–42.

(64) Patrignani P, Filabozzi P, Patrono C. Selective cumulative inhibition of platelet thromboxane production by low-dose aspirin in healthy subjects. J Clin Invest 1982;69:1366–72.

(65) FitzGerald GA, Oates JA, Hawiger J, Maas RL, Roberts LJ 2nd, Lawson JA, et al. Endogenous biosynthesis of prostacyclin and thromboxane and platelet function during chronic administration of aspirin in man. J Clin Invest 1983;71:678–88.

(66) Peterson WL, Cryer B. COX-1-sparing NSAIDs—is the enthusiasm justified? JAMA 1999;282:1961–3.

(67) Willoughby DA, Moore AR, Colville-Nash PR. COX-1, COX-2, and COX-3 and the future treatment of chronic inflammatory disease. Lancet 2000;355:646–8.

(68) Bombardier C, Laine L, Reicin A, Shapiro D, Burgos-Vargas R, Davis B, et al. Comparison of upper gastrointestinal toxicity of rofecoxib and naproxen in patients with rheumatoid arthritis. VIGOR Study Group. N Engl J Med 2000;343:1520–8.

(69) McAdam BF, Catella-Lawson F, Mardini I, Kapoor S, Lawson A, FitzGerald GA. Systemic biosynthesis of prostacyclin by cyclooxygenase (COX)-2: the human pharmacology of a selective inhibitor of COX-2 [erratum appears in Proc Natl Acad Sci U S A 1999;96:5890]. Proc Natl Acad Sci U S A 1999;96:272–7.

(70) Cullen L, Kelly L, Connor SO, Fitzgerald DJ. Selective cyclooxygenase-2 inhibition by nimesulide in man. J Pharmacol Exp Ther 1998;287:578–82.

(71) Catella-Lawson F, McAdam B, Morrison BW, Kapoor S, Kujubu D, Antes L, et al. Effects of specific inhibition of cyclooxygenase-2 on sodium balance, hemodynamics, and vasoactive eicosanoids. J Pharmacol Exp Ther 1999;289:735–41.

(72) Langman MJ, Jensen DM, Watson DJ, Harper SE, Zhao PL, Quan H, et al. Adverse upper gastrointestinal effects of rofecoxib compared with NSAIDs. JAMA 1999;282:1929–33.

(73) Torrance CJ, Jackson PE, Montgomery E, Kinzler KW, Vogelstein B, Wissner A, et al. Combinatorial chemoprevention of intestinal neoplasia. Nat Med 2000;6:1024–8.

(74) Gupta RA, DuBois RN. Combinations for cancer prevention. Nat Med 2000;6:974–5.

(75) Agarwal B, Rao CV, Bhendwal S, Ramey WR, Shirin H, Reddy BS, et al. Lovastatin augments sulindac-induced apoptosis in colon cancer cells and potentiates chemopreventive effect of sulindac. Gastroenterology 1999;117:838–47.

(76) Li H, Schut HA, Conran P, Kramer PM, Lubet RA, Steele VE, et al. Prevention by aspirin and its combination with α-difluoromethylornithine

of azoxymethane-induced tumors, aberrant crypt foci and prostaglandin E₂ levels in rat colon. Carcinogenesis 1999;20:425–30.

(77) Reddy BS, Nayini J, Tokumo K, Rigottgy K, Zang E, Kelloff G. Chemoprevention of colon carcinogenesis by concurrent administration of piroxicam, a nonsteroidal antiinflammatory drug with D,L-α-difluromethylornithine, an ornithine decarboxylase inhibitor, in diet. Cancer Res 1990;50:2562–8.

(78) Rao CV, Tokumo K, Rigotty J, Zang E, Kelloff G, Reddy BS. Chemoprevention of colon carcinogenesis by dietary administration of piroxicam α-difluoromethylornithine, 16 α-fluoro-5-androsten-17-one, and ellagic acid individually and in combination. Cancer Res 1991;51:4528–34.

(79) Rao CV, Rivenson A, Simi B, Zang E, Kelloff G, Steele V, et al. Chemoprevention of colon carcinogenesis by sulindac, a nonsteroidal anti-inflammatory agent. Cancer Res 1995;55:1464–72.

(80) Bennett A, Del Tacca M. Proceedings: Prostaglandins in human colonic carcinoma. Gut 1975;16:409.

(81) Jaffe BM. Prostaglandins and cancer: an update. Prostaglandins 1974;6:453–61.

(82) Pollard M, Zedeck MS. Induction of colon tumors in 1,2-dimethylhydrazine-resistant Lobund Wistar rats by methylazoxymethanol acetate. J Natl Cancer Inst 1978;61:493–4.

(83) Pollard M, Luckert PH. Indomethacin treatment of rats with dimethylhydrazine-induced intestinal tumors. Cancer Treat Rep 1980;64:1323–7.

(84) Pollard M, Luckert PH. Effect of indomethacin on intestinal tumors induced in rats by the acetate derivative of dimethylnitrosoamine. Science 1981;214:558–9.

(85) Pollard M, Luckert PH. Treatment of chemically-induced intestinal cancers with indomethacin. Proc Soc Exp Bio Med 1981;167:161–4.

(86) Pollard M, Luckert PH. Prolonged antitumor effect of indomethacin on autochthonous intestinal tumors in rats. J Natl Cancer Inst 1983;70:1103–5.

(87) Pollard M, Luckert PH, Schmidt MA. The suppressive effect of piroxicam on autochthonous intestinal tumors in the rat. Cancer Lett 1983;21:57–61.

(88) Pollard M, Luckert PH. Effect of piroxicam on primary intestinal tumors induced in rats by N-methylnitrosourea. Cancer Lett 1984;25:117–21.

(89) Pollard M. Antitumor effect of indomethacin in rats with autochthonous intestinal tumors. In: Levin B, Riddell R, editors. Frontiers in gastrointestinal cancer. New York (NY): Elsevier; 1984. p. 91–103.

(90) Pollard M, Luckert PH. Prevention and treatment of primary intestinal tumors in rats by piroxicam. Cancer Res 1989;49:6471–3.

(91) Kudo T, Narisawa T, Abo S. Antitumor activity of indomethacin on methylazoxymethanol-induced large bowel tumors in rats. Gann 1980;71:260–4.

(92) Narisawa T, Sato M, Tani M, Takahashi T, Goto A. Inhibition of development of methylnitrosourea-induced rat colon tumors by indomethacin treatment. Cancer Res 1981;41:1954–7.

(93) Narisawa T, Sato M, Sano M, Takahashi T. Inhibition of development of methylnitrosourea-induced rat colonic tumors by peroral administration of indomethacin. Gann 1982;73:377–81.

(94) Narisawa T, Satoh M, Sano M, Takahashi T. Inhibition of initiation and promotion by N-methylnitrosourea-induced colon carcinogenesis in rats by non-steroid anti-inflammatory agent indomethacin. Carcinogenesis 1983;4:1225–7.

(95) Narisawa T, Hermanek P, Habs M, Schmahl D. Reduction of carcinogenicity of N-nitrosomethylurea by indomethacin and failure of resuming effect of prostaglandin E₂ (PGE₂) against indomethacin. J Cancer Res Clin Oncol 1984;108:239–42.

(96) Metzger U, Meier J, Uhlschmid G, Weihe H. Influence of various prostaglandin synthesis inhibitors on DMH-induced rat colon cancer. Dis Colon Rectum 1984;27:366–9.

(97) Nigro ND, Bull AW, Boyd ME. Inhibition of intestinal carcinogenesis in rats: effect of difluoromethylornithine with piroxicam or fish oil. J Natl Cancer Inst 1986;77:1309–13.

(98) Reddy BS, Maruyama H, Kelloff G. Dose-related inhibition of colon carcinogenesis by dietary piroxicam, a nonsteroidal antiinflammatory drug, during different stages of rat colon tumor development. Cancer Res 1987;47:5340–6.

(99) Reddy BS, Tokumo K, Kulkarni N, Aligia C, Kelloff G. Inhibition of colon carcinogenesis by prostaglandin synthesis inhibitors and related compounds. Carcinogenesis 1992;13:1019–23.

(100) Reddy BS, Rao CV, Rivenson A, Kelloff G. Inhibitory effect of aspirin on azoxymethane-induced colon carcinogenesis in F344 rats. Carcinogenesis 1993;14:1493–7.

(101) Craven PA, DeRubertis FR. Effects of aspirin on 1,2-dimethylhydrazine-induced colonic carcinogenesis. Carcinogenesis 1992;13:541–6.

(102) Moorghen M, Ince P, Finney KJ, Sunter JP, Appleton DR, Watson AJ. A protective effect of sulindac against chemically-induced primary colonic tumours in mice. J Pathol 1988;156:341–7.

(103) Moorghen M, Ince P, Finney KJ, Sunter JP, Watson AJ, Appleton DR. The effect of sulindac on colonic tumor formation in dimethylhydrazine-treated mice. Acta Histochem Suppl 1990;39:195–9.

(104) Skinner SA, Penney AG, O'Brien P. Sulindac inhibits the rate of growth and appearance of colon tumors in the rat. Arch Surg 1991;126:1094–6.

(105) Davis AE, Patterson F. Aspirin reduces the incidence of colonic carcinoma in the dimethydrazine rat animal model. Aust N Z J Med 1994;24:301–3.

(106) Hursting S, Velasco M, Woods C, Wang K, Wargovich M. Chemoprevention of azoxmethane-induced aberrant crypt foci and colon tumors in p53-deficient mice by sulindac [abstract]. Bethesda (MD): Am Soc Prev Oncol; 1998.

(107) Kawamori T, Rao CV, Seibert K, Reddy BS. Chemopreventive activity of celecoxib, a specific cyclooxygenase-2 inhibitor, against colon carcinogenesis. Cancer Res 1998;58:409–12.

(108) Li H, Kramer P, Steele V, Kelloff G, Lubet R, Pereira M. Effect of duration of treatment with piroxicam on azoxymethane-induced colon cancer, aberrant crypt foci, apoptosis and cell proliferation in rats [abstract]. Proc Am Assoc Cancer Res 1998;39:23.

(109) Barnes CJ, Lee M. Determination of an optimal dosing regimen for aspirin chemoprevention of 1,2-dimethylhydrazine-induced colon tumours in rats. Br J Cancer 1999;79:1646–50.

(110) Reddy BS, Kawamori T, Lubet RA, Steele VE, Kelloff GJ, Rao CV. Chemopreventive efficacy of sulindac sulfone against colon cancer depends on time of administration during carcinogenic process. Cancer Res 1999;59:3387–91.

(111) Piazza GA, Alberts DS, Hixson LJ, Paranka NS, Li H, Finn T, et al. Sulindac sulfone inhibits azoxymethane-induced colon carcinogenesis in rats without reducing prostaglandin levels. Cancer Res 1997;57:2909–15.

(112) Mereto E, Frencia L, Ghia M. Effect of aspirin on incidence and growth of aberrant cypt foci induced in the rat colon by 1,2-dimethylhydrazine. Cancer Lett 1994;76:5–9.

(113) Wargovich MJ, Chen CD, Harris C, Yang E, Velasco M. Inhibition of aberrant crypt growth by non-steroidal anti-inflammatory agents and differentiation agents in the rat colon. Int J Cancer 1995;60:515–9.

(114) Reddy BS, Rao CV, Seibert K. Evaluation of cyclooxygenase-2 inhibitor for potential chemopreventative properties in colon carcinogenesis. Cancer Res 1996;56:4566–9.

(115) Pereira MA, Barnes LH, Steele VE, Kelloff GV, Lubet RA. Piroxicam-induced regression of azoxymethane-induced aberrant crypt foci and prevention of colon cancer in rats. Carcinogenesis 1996;17:373–6.

(116) Takahashi M, Fukutake M, Yokota S, Ishida K, Wakabayashi K, Sugimura T. Suppression of azoxymethane-induced aberrant crypt foci in rat colon by nimesulide, a selective inhibitor of cyclooxygenase 2. J Cancer Res Clin Oncol 1996;122:219–22.

(117) Weisburger J, Reddy B, Joftes D. Colorectal cancer. International Union Against Cancer (UICC) Technical Report 19. Geneva (Switzerland): UICC; 1975.

(118) Reddy BS, Hirose Y, Lubet R, Steele V, Kelloff G, Paulson S, et al. Chemoprevention of colon cancer by specific cyclooxygenase-2 inhibitor, celecoxib, administered during different stages of carcinogenesis. Cancer Res 2000;60:293–7.

(119) Botha JH, Robinson KM, Ramchurren N, Reddi K, Norman RJ. Human esophageal carcinoma cell lines: prostaglandin production, biological properties, and behavior in nude mice. J Natl Cancer Inst 1986;76:1053–6.

(120) Carlton P, Gopalakrishnan R, Stoner G. Expression of cyclooxygenase-1 and cyclooxygenase-2 in NMBA-induced rat esophageal tumorigenesis [abstract]. Proc Am Assoc Cancer Res 1999;40:abstract 2378.

(121) Li M, Lotan R, Levin B, Tahara E, Lippman SM, Xu XC. Aspirin induction of apoptosis in esophageal cancer: a potential for chemoprevention. Cancer Epidemiol Biomarkers Prev 2000;9:545–9.

(122) Lehnert T, Deschner EE, Karmali RA, DeCosse JJ. Effect of flurbiprofen

and 16,16-dimethyl-prostaglandin E_2 on gastrointestinal tumorigenesis induced by *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine in rats. I. Squamous epithelium and mesenchymal tissue. J Natl Cancer Inst 1987;78:923–9.

(123) Shibata MA, Hirose M, Masuda A, Kato T, Mutai M, Ito N. Modification of BHA forestomach carcinogenesis in rats: inhibition by diethylmaleate or indomethacin and enhancement by a retinoid. Carcinogenesis 1993;14: 1265–9.

(124) Fischer SM, Lo HH, Gordon GB, Seibert K, Kelloff G, Lubet RA, et al. Chemopreventive activity of celecoxib, a specific cyclooxygenase-2 inhibitor, and indomethacin against ultraviolet light-induced skin carcinogenesis. Mol Carcinog 1999;25:231–40.

(125) Rozic JG, Chakraborty C, Lala PK. Cyclooxygenase inhibitors retard murine mammary tumor progression by reducing tumor cell migration, invasiveness and angiogenesis. Int J Cancer 2001;93:497–506.

(126) Liu CH, Chang SH, Narko K, Trifan OC, Wu MT, Smith E, et al. Overexpression of cyclooxygenase-2 is sufficient to induce tumorigenesis in transgenic mice. J Biol Chem 2001;276:18563–9.

(127) Alshafie GA, Abou-Issa HM, Seibert K, Harris RE. Chemotherapeutic evaluation of Celecoxib, a cyclooxygenase-2 inhibitor, in a rat mammary tumor model. Oncol Rep 2000;7:1377–81.

(128) Nakatsugi S, Ohta T, Kawamori T, Mutoh M, Tanigawa T, Watanabe K, et al. Chemoprevention by nimesulide, a selective cyclooxygenase-2 inhibitor, of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP)-induced mammary gland carcinogenesis in rats. Jpn Cancer Res 2000;91: 886–92.

(129) Yao R, Rioux N, Castonguay A, You M. Inhibition of COX-2 and induction of a apoptosis: two determinants of nonsteroidal and anti-inflammatory drugs' chemopreventive efficacies in mouse lung tumorigenesis. Exp Lung Res 2000;26:731–42.

(130) Tsubouchi Y, Mukai S, Kawahito Y, Yamada R, Kohno M, Inoue K, et al. Meloxicam inhibits the growth of non-small cell lung cancer. Anticancer Res 2000;20:2867–72.

(131) Duperron C, Castonguay A. Chemoprevention efficacies of aspirin and sulindac against lung tumorigenesis in A/J mice. Carcinogenesis 1997;18: 1001–6.

(132) Rioux N, Castonguay A. Prevention of NNK-induced lung tumorigenesis in A/J mice by acetylsalicylic acid and NS-398. Cancer Res 1998;58: 5354–60.

(133) Tremblay C, Dore M, Bochsler P, Sirois J. Induction of prostaglandin G/H synthase-2 in a canine model of spontaneous prostatic adenocarcinoma. J Natl Cancer Inst 1999;91:1398–403.

(134) Liu XH, Kirschenbaum A, Yao S, Lee R, Holland JF, Levine AC. Inhibition of cyclooxygenase-2 suppresses angiogenesis and the growth of prostate cancer *in vivo*. J Urol 2000;164(3 Pt 1):820–5.

(135) Okajima A, Denda A, Ozono S, Takahama M, Akai H, Sasaki Y, et al. Chemopreventive effects of nimesulide, a selective cyclooxygenase-2 inhibitor, on the development of rat urinary bladder carcinomas initiated by *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine. Cancer Res 1998;58:3028–31.

(136) Grubbs CJ, Lubet RA, Koki AT, Leahy KM, Masferrer JL, Steele VE, et al. Celecoxib inhibits *N*-butyl-*N*-(4-hydroxybutyl)-nitrosamine-induced urinary bladder cancers in male B6D2F1 mice and female Fischer-344 rats. Cancer Res 2000;60:5599–602.

(137) Klan R, Knispel H, Meier T. Acetylsalicylic acid inhibition of *N*-butyl-(4-hydroxybutyl)nitrosamine-induced bladder carcinogenesis in rats. J Cancer Res Clin Oncol 1993;119:482–5.

(138) Giardiello FM. NSAID-induced polyp regression in familial adenomatous polyposis patients. Gastroenterol Clin North Am 1996;25:349–62.

(139) FDA Center for Drug Evaluation and Research. New drug approvals - Celebrex. Available from: www.fda.gov/cder/foi/label/1999/ 21156lbl.pdf; 1999.

(140) Lynch HT, Thorson AG, Smyrk T. Rectal cancer after prolonged sulindac chemoprevention. A case report. Cancer 1995;75:936–8.

(141) Tonelli F, Valanzano R. Sulindac in familial adenomatous polyposis [letter]. Lancet 1993;342:1120.

(142) Ladenheim J, Garcia G, Titzer D, Herzenberg H, Lavori P, Edson R, et al. Effect of sulindac on sporadic colonic polyps. Gastroenterology 1995; 108:1083–7.

(143) Kensler TW, Tsuda H, Wogan GN. United States–Japan workshop on New Rodent Models for the Analysis and Prevention of Carcinogenesis. Cancer Epidemiol Biomarkers Prev 1999;8:1033–7.

(144) Jacoby RF, Seibert K, Cole CE, Kelloff G, Lubet RA. The cyclooxygenase-2 inhibitor celecoxib is a potent preventive and therapeutic agent in the min mouse model of adenomatous polyposis. Cancer Res 2000;60: 5040–4.

(145) Boolbol SK, Dannenberg AJ, Chadburn A, Martucci C, Guo XJ, Ramonetti JT, et al. Cyclooxygenase-2 overexpression and tumor formation are blocked by sulindac in a murine model of familial adenomatous polyposis. Cancer Res 1996;56:2556–60.

(146) Chiu CH, McEntee MF, Whelan J. Sulindac causes rapid regression of preexisting tumors in Min/+ mice independent of prostaglandin biosynthesis. Cancer Res 1997;57:4267–73.

(147) Mahmoud NN, Bilinski RT, Churchill MR, Edelmann W, Kucherlapati R, Bertagnoli MM. Genotype–phenotype correlation in murine Apc mutation: differences in enterocyte migration and response to sulindac. Cancer Res 1999;59:353–9.

(148) Mahmoud NN, Dannenberg AJ, Mestre J, Bilinski RT, Churchill MR, Martucci C, et al. Aspirin prevents tumors in a murine model of familial adenomatous polyposis. Surgery 1998;124:225–31.

(149) Oshima M, Murai N, Kargman S, Arguello M, Luk P, Kwong E, et al. Chemoprevention of intestinal polyposis in the Apc$^{(delta)716}$ mouse by rofecoxib, a specific cyclooxygenase-2 inhibitor. Cancer Res 2001;61: 1733–40.

(150) Thun MJ. Aspirin, NSAIDs, and digestive tract cancers. Cancer Metastasis Rev 1994;13:269–77.

(151) Thun M, Hennekens C. Aspirin and other nonsteroidal antiinflammatory drugs and the risk of cancer development. In: DeVita VT Jr, Hellman S, Rosenberg S, editors. Cancer: principles & practice of oncology. Philadelphia (PA): Lippincott Williams & Wilkins; 2001. p. 601–10.

(152) Thun MJ, Heath CW Jr. Aspirin use and reduced risk of gastrointestinal tract cancers in the American Cancer Society prospective studies. Prev Med 1995;24:116–8.

(153) Friedman GD, Ury K. Initial screening for carcinogenicity of commonly used drugs. J Natl Cancer Inst 1980;65:723–33.

(154) Egan KM, Stampfer MJ, Giovannucci E, Rosner BA, Colditz GA. Prospective study of regular aspirin use and the risk of breast cancer. J Natl Cancer Inst 1996;88:988–93.

(155) Harris RE, Kasbari S, Farrar WB. Prospective study of nonsteroidal anti-inflammatory drugs and breast cancer. Oncol Rep 1999;6:71–3.

(156) Sharpe CR, Collet JP, McNutt M, Belzile E, Boivin JF, Hanley JA. Nested case–control study of the effects of non-steroidal anti-inflammatory drugs on breast cancer risk and stage. Br J Cancer 2000;83:112–20.

(157) Khuder SA, Mutgi AB. Breast cancer and NSAID use: a meta-analysis. Br J Cancer 2001;84:1188–92.

(158) Norrish AE, Jackson RT, McRae CU. Non-steroidal anti-inflammatory drugs and prostate cancer progression. Int J Cancer 1998;77:511–5.

(159) Cramer DW, Harlow B, Titus-Ernstoff L, Bohlke K, Welch WR, Greenberg ER. Over-the-counter analgesics and risk of ovarian cancer. Lancet 1998;351:104–7.

(160) Rodriguez C, Henley SJ, Calle EE, Thun MJ. Paracetamol and risk of ovarian cancer mortality in a prospective study of women in the USA [letter]. Lancet 1998;352:1354–5.

(161) Tavani A, Gallus S, La Vecchia C, Conti E, Montella M, Franceschi S. Aspirin and ovarian cancer: an Italian case–control study. Ann Oncol 2000;11:1171–3.

(162) Tsujii M, DuBois RN. Alterations in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandin endoperoxide synthase 2. Cell 1995;83:493–501.

(163) He TC, Chan TA, Vogelstein B, Kinzler KW. PPARdelta is an APC-regulated target of nonsteroidal anti-inflammatory drugs. Cell 1999;99: 335–45.

(164) Afford S, Randhawa S. Apoptosis. Mol Pathol 2000;53:55–63.

(165) Morin PJ, Vogelstein B, Kinzler KW. Apoptosis and APC in colorectal tumorigenesis. Proc Natl Acad Sci U S A 1996;93:7950–4.

(166) Kinzler KW, Vogelstein B. Colorectal tumors. In: Vogelstein B, Kinzler K, editors. The genetic basis of human cancer. New York (NY): McGraw-Hill; 1998. p. 565–87.

(167) Bedi A, Pasricha PJ, Akhtar AJ, Barber JP, Bedi GC, Giardiello FM, et al. Inhibition of apoptosis during development of colorectal cancer. Cancer Res 1995;55:1811–6.

(168) Pasricha PJ, Bedi A, O'Connor K, Rashid A, Akhtar AJ, Zahurak MV, et

al. The effects of sulindac on colorectal proliferation and apoptosis in familial adenomatous polyposis. Gastroenterology 1995;109:994–8.

(169) Jacobasch G, Schmehl K, Schmidt U. Influence of meloxicam on loss of apoptotic activity during carcinogenesis [abstract]. Proc Am Assoc Cancer Res 1998;39:197.

(170) Samaha H, Kelloff G, Steele V, Rao C, Reddy BS. Modulation of apoptosis by sulindac, curcumin, phenylethyl-3-methylcaffeate, and 6-phenyl-hexyl isothiocyanate: apoptotic index as a biomarker in colon cancer chemoprevention and promotion. Cancer Res 1997;57:1301–5.

(171) Uefuji K, Ichikura T, Shinomiya N, Mochizuki H. Induction of apoptosis by JTE-522, a specific cyclooxygenase-2 inhibitor, in human gastric cancer cell lines. Anticancer Res 2000;20:4279–84.

(172) Souza RF, Shewmake K, Beer DG, Cryer B, Spechler SJ. Selective inhibition of cyclooxygenase-2 suppresses growth and induces apoptosis in human esophageal adenocarcinoma cells. Cancer Res 2000;60:5767–72.

(173) Sumitani K, Kamijo R, Toyoshima T, Nakanishi Y, Takizawa K, Hatori M, et al. Specific inhibition of cyclooxygenase-2 results in inhibition of proliferation of oral cancer cell lines via suppression of prostaglandin E2 production. J Oral Pathol Med 2001;30:41–7.

(174) Joki T, Heese O, Nikas DC, Bello L, Zhang J, Kraeft SK, et al. Expression of cyclooxygenase 2 (COX-2) in human glioma and *in vitro* inhibition by a specific COX-2 inhibitor, NS-398. Cancer Res 2000;60:4926–31.

(175) Molina MA, Sitja-Arnau M, Lemoine MG, Frazier ML, Sinicrope FA. Increased cyclooxygenase-2 expression in human pancreatic carcinomas and cell lines: growth inhibition by nonsteroidal anti-inflammatory drugs. Cancer Res 1999;59:4356–62.

(176) Marx J. Cancer research. Anti-inflammatories inhibit cancer growth—but how? [news] Science 2001;291:581–2.

(177) DuBois RN, Shao J, Tsujii M, Sheng H, Beauchamp RD. G₁ delay in cells overexpressing prostaglandin endoperoxide synthase-2. Cancer Res 1996; 56:733–7.

(178) Elder DJ, Halton DE, Hague A, Paraskeva C. Induction of apoptotic cell death in human colorectal carcinoma cell lines by a cyclooxygenase-2 (COX-2)-selective nonsteroidal anti-inflammatory drug: independence from COX-2 protein. Clin Cancer Res 1997;3:1679–83.

(179) Shiff SJ, Koutsos MI, Qiao L, Rigas B. Nonsteroidal antiinflammatory drugs inhibit the proliferation of colon adenocarcinoma cells: effects on cell cycle and apoptosis. Exp Cell Res 1996;222:179–88.

(180) Shiff SJ, Qiao L, Tsai LL, Rigas B. Sulindac sulfide, an aspirin-like compound, inhibits proliferation, causes cell cycle quiescence, and induces apoptosis in HT-29 colon adenocarcinoma cells. J Clin Invest 1995; 96:491–503.

(181) Eberhart CE, Coffey RJ, Radhika A, Giardiello FM, Ferrenbach S, DuBois RN. Up-regulation of cyclooxgenase 2 gene expression in human colorectal adenomas and adenocarcinomas. Gastroenterology 1994;107: 1183–8.

(182) Fujita T, Matsui M, Takaku K, Uetake H, Ichikawa W, Taketo MM, et al. Size- and invasion-dependent increase in cyclooxygenase 2 levels in human colorectal carcinomas. Cancer Res 1998;58:4823–6.

(183) Langenbach R, Morham SG, Tiano HF, Loftin CD, Ghanayem BI, Chulada PC, et al. Prostaglandin synthase 1 gene disruption in mice reduces arachidonic acid-induced inflammation and indomethacin-induced gastric ulceration. Cell 1995;83:483–92.

(184) Langenbach R, Loftin C, Lee C, Tiano H. Cyclooxygenase knockout mice: models for elucidating isoform-specific functions. Biochem Pharmacol 1999;58:1237–46.

(185) Patrono C, Patrignani P, Garcia Rodriguez LA. Cyclooxygenase-selective inhibition of prostanoid formation: transducing biochemical selectivity into clinical read-outs. J Clin Invest 2001;108:7–13.

(186) Vane JR. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nat New Biol 1971;231:232–5.

(187) Cao Y, Pearman AT, Zimmerman GA, McIntyre TM, Prescott SM. Intracellular unesterified arachidonic acid signals apoptosis. Proc Natl Acad Sci U S A 2000;97:11280–5.

(188) Chan TA, Morin PJ, Vogelstein B, Kinzler KW. Mechanisms underlying nonsteroidal antiinflammatory drug-mediated apoptosis. Proc Natl Acad Sci U S A 1998;95:681–6.

(189) Piazza GA, Rahm AL, Krutzsch M, Sperl G, Paranka NS, Gross PH, et al. Antineoplastic drugs sulindac sulfide and sulfone inhibit cell growth by inducing apoptosis. Cancer Res 1995;55:3110–6.

(190) He TC, Sparks AB, Rago C, Hermeking H, Zawel L, da Costa LT, et al. Identification of c-MYC as a target of the APC pathway. Science 1998; 281:1509–12.

(191) Kopp E, Ghosh S. Inhibition of NF-kappa B by sodium salicylate and aspirin. Science 1994;265:956–8.

(192) Yamamoto Y, Yin MJ, Lin KM, Gaynor RB. Sulindac inhibits activation of the NF-kappaB pathway. J Biol Chem 1999;274:27307–14.

(193) Schwenger P, Bellosta P, Vietor I, Basilico C, Skolnik EY, Vilcek J. Sodium salicylate induces apoptosis via p38 mitogen-activated protein kinase but inhibits tumor necrosis factor-induced c-Jun N-terminal kinase/stress-activated protein kinase activation. Proc Natl Acad Sci U S A 1997;94:2869–73.

(194) Schwenger P, Alpert D, Skolnik EY, Vilcek J. Activation of p38 mitogen-activated protein kinase by sodium salicylate leads to inhibition of tumor necrosis factor-induced IkappaB alpha phosphorylation and degradation. Mol Cell Biol 1998;18:78–84.

(195) Wu GD. A nuclear receptor to prevent colon cancer. N Engl J Med 2000;342:651–3.

(196) Shureiqi I, Chen D, Lotan R, Yang P, Newman RA, Fischer SM, et al. 15-Lipoxygenase-1 mediates nonsteroidal anti-inflammatory drug-induced apoptosis independently of cyclooxygenase-2 in colon cancer cells. Cancer Res 2000;60:6846–50.

(197) Tsujii M, Kawano S, Tsuji S, Sawaoka H, Hori M, DuBois RN. Cyclooxygenase regulates angiogenesis induced by colon cancer cells. Cell 1998;93:705–16.

(198) Jones MK, Wang H, Peskar BM, Levin E, Itani RM, Sarfeh IJ, et al. Inhibition of angiogenesis by nonsteroidal anti-inflammatory drugs: insight into mechanisms and implications for cancer growth and ulcer healing. Nat Med 1999;5:1418–23.

(199) Masferrer JL, Isakson PC, Seibert K. Cyclooxygenase-2 inhibitors: a new class of anti-inflammatory agents that spare the gastrointestinal tract. Gastroenterol Clin North Am 1996;25:363–72.

(200) Holash J, Maisonpierre PC, Compton D, Boland P, Alexander CR, Zagzag D, et al. Vessel cooption, regression, and growth in tumors mediated by angiopoietins and VEGF. Science 1999;284:1994–8.

(201) Masferrer JL, Leahy KM, Koki AT, Zweifel BS, Settle SL, Woerner BM, et al. Antiangiogenic and antitumor activities of cyclooxygenase-2 inhibitors. Cancer Res 2000;60:1306–11.

(202) Williams CS, Tsujii M, Reese J, Dey SK, DuBois RN. Host cyclooxygenase-2 modulates carcinoma growth. J Clin Invest 2000;105:1589–94.

(203) Ruffin MT 4th, Krishnan K, Rock CL, Normolle D, Vaerten MA, Peters-Golden M, et al. Suppression of human colorectal mucosal prostaglandins: determining the lowest effective aspirin dose. J Natl Cancer Inst 1997; 89:1152–60.

(204) Carbone PP, Douglas JA, Larson PO, Verma AK, Blair IA, Pomplun M, et al. Phase I chemoprevention study of piroxicam and alpha-difluoromethylornithine. Cancer Epidemiol Biomarkers Prev 1998;7: 907–12.

(205) Calaluce R, Earnest DL, Heddens D, Einspahr JG, Roe D, Bogert CL, et al. Effects of piroxicam on prostaglandin E2 levels in rectal mucosa of adenomatous polyp patients: a randomized phase IIb trial. Cancer Epidemiol Biomarkers Prev 2000;9:1287–92.

(206) Chow HH, Earnest DL, Clark D, Mason-Liddil N, Kramer CB, Einspahr JG, et al. Effect of subacute ibuprofen dosing on rectal mucosal prostaglandin E2 levels in healthy subjects with a history of resected polyps. Cancer Epidemiol Biomarkers Prev 2000;9:351–6.

(207) Harris RE, Alshafie G, Abou-Issa H, Seibert K. Chemoprevention of breast cancer in rats by celecoxib, a cyclooxygenase 2 inhibitor. Cancer Res 2000;60:2101–3.

(208) Ristimaki A, Honkanen N, Jankala H, Sipponen P, Harkonen M. Expression of cyclooxygenase-2 in human gastric carcinoma. Cancer Res 1997; 57:1276–80.

(209) Mohammed SI, Knapp DW, Bostwick DG, Foster RS, Khan KN, Masferrer JL, et al. Expression of cyclooxygenase-2 (COX-2) in human invasive transitional cell carcinoma (TCC) of the urinary bladder. Cancer Res 1999;59:5647–50.

(210) Kokawa A, Kondo H, Gotoda T, Ono H, Saito D, Nakadaira S, et al. Increased expression of cyclooxygenase-2 in human pancreatic neoplasms and potential for chemoprevention by cyclooxygenase inhibitors. Cancer 2001;91:333–8.

(211) Castelao JE, Yuan JM, Gago-Dominguez M, Yu MC, Ross RK. Non-steroidal anti-inflammatory drugs and bladder cancer prevention. Br J Cancer 2000;82:1364–9.

(212) Williams CS, Watson AJ, Sheng H, Helou R, Shao J, DuBois RN. Celecoxib prevents tumor growth *in vivo* without toxicity to normal gut: lack of correlation between *in vitro* and *in vivo* models. Cancer Res 2000;60: 6045–51.

(213) Moore RJ, Zweifel BS, Heuvelman DM, Leahy KM, Edwards DA, Woener BM, et al. Enhanced antitumor activity by co-administration of celecoxib and the chemotherapeutic agents cyclophosphamide and 5-FU [abstract]. Proc Am Assoc Cancer Res 2000;41:409.

(214) Milas L, Kishi K, Hunter N, Mason K, Masferrer JL, Tofilon PJ. Enhancement of tumor response to gamma radiation by an inhibitor of cyclooxygenase-2 enzymeb. J Natl Cancer Inst 1999;91:1501–4.

(215) Sheehan KM, Sheahan K, O'Donoghue DP, MacSweeney F, Conroy RM, Fitzgerald DJ, et al. The relationship between cyclooxygenase-2 expression and colorectal cancer. JAMA 1999;282:1254–7.

(216) Prescott SM. Is cyclooxygenase-2 the alpha and the omega in cancer? J Clin Invest 2000;105:1511–3.

(217) Hong WK, Spoon MB. Recent advances in chemoprevention of cancer. Science 1997;278:1073–7.

(218) Gibbs JB. Mechanism-based target identification and drug discovery in cancer research. Science 2000;287:1969–74.

NOTES