GASTROENTEROLOGY 2004;126:1423–1447

# SPECIAL REPORTS AND REVIEWS

## Colorectal Cancer Chemoprevention—An Overview of the Science

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**The development and dissemination of sophisticated detection technologies have recently exposed the high prevalence of preinvasive colorectal neoplasia in the adult U.S. population. Although cancer screening and surveillance provide opportunities for risk stratification, they achieve risk reduction only when coupled with effective interventions. This review surveys the lead compounds for colorectal cancer prevention and the measures by which they may be prioritized for clinical testing. Clinical trials remain the rate-limiting step in agent development, and novel trial designs are needed to hasten agent identification and testing for cancer prevention. Innovative research models include the nesting of prevention end points within cancer treatment trials and within trials testing promising preventive compounds intended for nononcologic indications.**

Colorectal cancer (CRC) is a major health concern, with more than 944,000 cases and 492,000 cancer-related deaths reported worldwide in 2000.[1] In the United States, CRC affects nearly 150,000 people each year, with as many as 56,730 deaths anticipated in 2004.[2] These statistics are sobering, considering that in most instances CRC should be preventable by well-established screening and surgical techniques. Nevertheless, CRC death rates remain unacceptably high, calling for better compliance with screening guidelines and the identification of complementary strategies to reduce the public health burden of this disease.

One promising strategy, cancer chemoprevention, is based on understanding the molecular determinants of cancer. Cancer chemoprevention strives to block, reverse, or delay carcinogenesis before the development of invasive disease by targeting key molecular derangements using pharmacological or nutritional agents.[3] Chemopreventive interventions may be applied at any time during carcinogenesis, from the initial molecular defect through the accumulated molecular, cellular, and histopathologic aberrations that characterize disease progression before an invasive—and potentially metastatic—stage. In most instances, CRC develops over 10–20 years, providing time for disease identification and interruption long before it poses a clinical threat.[4]

### Mechanisms Underlying Colorectal Carcinogenesis—The Targets of Molecular Prevention

Carcinogenesis results from an interplay between environmental factors and susceptibility genes that sets off a complex series of neoplastic events. Malignant transformation of the colorectal epithelium typically occurs as a multistep, multipath, multifocal process that requires sequential or concomitant damage to several genes within and across cellular generations. Derangements of key molecular processes, including disruption of genomic fidelity, signal transduction, cell division, apoptosis, angiogenesis, and compartmental boundaries (e.g., tissue invasion and metastasis), contribute to neoplastic progression.[5] These variations explain how cells within an epithelium sharing common genetic origins and environments may display different degrees of neoplastic change.

Genetic and epigenetic alterations underlying colorectal neoplasia are most likely stochastic events that drive neoplastic progression.[6] At present, higher-order biomarkers (tissue > cellular > protein > RNA > DNA) allow for more confident predictions of cancer risk and guidance for the application of preventive interventions. At the cellular or tissue levels, hallmarks of preinvasive neoplasia include (1) a relative imbalance between cryp-

*Abbreviations used in this paper:* ACF, aberrant crypt foci; CI, confidence interval; COX, cyclooxygenase; COXIB, cyclooxygenase inhibitor; CRC, colorectal cancer; EGFR, epidermal growth factor receptor; FAP, familial adenomatous polyposis; 5-FU, 5-fluorouracil; HR, hazard ratio; IEN, intraepithelial neoplasia; PPAR, peroxisome proliferator–activated receptor; RR, relative risk; TI, therapeutic index.

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0016-5085/04/$0.00
doi:10.1053/j.gastro.2004.03.002

tal proliferation and apoptosis, (2) morphological alterations that are collectively termed atypia or dysplasia, and (3) neoangiogenesis. Cryptal hyperplasia, a proliferation of normal-appearing cells, commonly results from cumulative genetic (e.g., mutations in *APC* or *Bcl*-2 or c-*myc* overexpression) and epigenetic (e.g., methylation) changes in pathways that regulate cell cycle progression or apoptosis.[7] Later-stage neoplastic transition is evident in crypts that contain larger cells with atypia characterized by abnormal nuclear and/or cellular shapes (i.e., aberrant crypt foci; ACF) that efface normal-appearing crypts, often in the context of k-*ras* mutations.[8] The evolution from ACF to adenoma and later to carcinoma is typically associated with aberrations of SMAD-2 and -4, DCC, and p53.[9] Cumulative molecular defects within phenotypically normal mucosa or those expressed as higher-level pathologies (e.g., ACF and adenomas) represent an even greater risk for CRC.[10] The accessibility of the colorectum to prospective and serial observation, biopsy, and intervention simplifies investigations into the natural history of these events and their response to chemopreventive compounds.

## Advancing Molecular Prevention Through Clinical Research

Carcinogenesis is typically a slow, chronic process, and the development of invasive disease is characterized by prototypical molecular derangements. This potentially provides time and a wealth of molecular targets for cancer prevention, but it challenges efforts to show agent efficacy in a reasonably efficient, reliable, and ethical manner. Reductions in cancer incidence or mortality are appealing trial end points because of their irrefutable clinical significance. However, cancer incidence and mortality would be expected to be relatively uncommon if screening, surveillance, and surgical resection (including endoscopic polypectomy, as appropriate) were performed in a manner consistent with standard care. As a result, demonstration of further reductions in cancer incidence or mortality through the use of chemopreventive agents would require relatively large, long, costly, and complicated clinical testing. For this reason, and because adenomas are early neoplastic lesions as well as markers of risk, CRC chemoprevention trials typically focus on reductions in adenomas.

Although specific aspects of adenoma reduction with regard to CRC prevention are still debated,[11] dozens of preclinical, epidemiological, and experimental studies support colorectal adenomas as reasonable clinical end points for chemoprevention trials.[12] First, adenomas share many molecular derangements common to CRC, suggesting a common molecular etiology. Second, adenomas and CRC share several risk factors (e.g., age, personal or family history of colorectal neoplasia, and dietary factors). Third, adenomas are viewed as reliable surrogates for CRC incidence and mortality in the context of surgical interventions. For example, the clinical benefits of adenomectomy were prospectively evaluated in the National Polyp Study, which observed 2632 patients with prior adenomas randomized to undergo surveillance colonoscopy with or without paired barium enema examinations at intervals of 1 or 3 years.[13,14] Over the course of this study, adenoma detection rates averaged 10% per year. Advanced lesions characterized by their larger size (>1.0 cm), high grade, or villous histology occurred at a tenth of this rate but represent more worrisome pathologies. Winawer et al.[13,14] reported relative reductions in CRC incidence of 76%–90%, compared with 3 historical cohorts from St. Mark's, Mayo, and Surveillance, Epidemiology, and End Results, providing support for the identification and treatment of adenomas as an effective strategy for cancer prevention. The magnitude of this benefit must be viewed cautiously, however, because of the challenges in comparing data across different cohorts over time. Even so, subsequent observational and experimental studies confirm significant reductions in CRC mortality and, in some cases, incidence, attributable to adenomectomy and/or surgical excision of lesions detected through screening.[15–21] Fourth, in carcinogen-driven animal models, chemopreventive compounds typically achieve similar effects against adenomas and carcinomas.[22] Fifth, correlations between chemopreventive effects on adenomas, cancer, and cancer-associated mortality have been shown by a large and persuasive database of studies showing that aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) are associated with consistent reductions in disease across the entire spectrum of colorectal neoplasia (e.g., ACF, adenomas, CRC, and CRC-associated mortality).[23,24] Sixth, although most adenomas do not progress to cancer, nearly all cancers seem to arise from adenomas.[25] As a result, colorectal adenomas are considered a disease worthy of screening, surveillance, intervention, and reimbursement according to national standards of clinical practice.[26] Finally, adenomas have been practically regarded as acceptable end points for agent development in CRC chemoprevention by practitioners in the field for more than a decade.[27,28] The US Food and Drug Administration recently considered adenomas as reasonable end points for chemoprevention research by granting provisional approval of celecoxib, a

cyclooxygenase (COX)-2 inhibitor (COXIB), as an adjunct to standard surveillance and surgical prophylaxis in individuals with familial adenomatous polyposis (FAP) (see http://www.fda.gov/cder/approval/index.htm).

The development of compounds to treat intraepithelial neoplasia (IEN), such as adenomas, represents a significant conceptual advance in clinical cancer prevention research. An American Association of Cancer Research working group report recently summarized the evidence linking IEN modulation to reductions in cancer incidence and mortality, among other clinical benefits.[12] Indeed, the acceptance of IEN modulation as clinically beneficial has already led to a decade of drug discovery and approval in other neoplastic conditions (e.g., preinvasive bladder neoplasia and actinic keratoses).[10] In March 2002, the Food and Drug Administration convened a meeting of their Gastrointestinal Drugs Advisory Committee to seek advice from independent experts regarding the review and approval of applications for chemopreventive compounds designed to treat or prevent adenomas (http://www.fda.gov/ohrms/dockets/ac/02/transcripts/3845t1.htm). Although no definitive guidelines were established per se, the group offered key recommendations for clinical trials intended to show chemopreventive drug efficacy and safety. General consensus was achieved on the following points: (1) adenomas may serve as meaningful end points in cancer prevention trials, with relative reductions of 25%–35% in adenoma number representing a reasonable lower threshold for efficacy; (2) trials should include supportive data on increases in the number of adenoma-free patients and/or reductions in adenoma size, histopathologic grade, or molecular aberrancy; (3) ideally, trials should be well controlled, ensure adequate compliance, and last 3 to 6 years; and (4) issues of safety, tachyphylaxis, and rebound should be addressed to ensure adequate characterization of test agents.

Once clinical value is shown, chemopreventive agents may be applied across a wide range of clinical scenarios, depending on agent efficacy and safety, as well as underlying cohort susceptibilities. Trials involving individuals with high-risk conditions typically require smaller cohorts observed over shorter periods of time, as compared with trials conducted in cohorts with lower susceptibility to cancer. For example, individuals with a personal history of colorectal neoplasia (e.g., colorectal adenoma or CRC) are at approximately 2–3-fold increased risk for developing metachronous neoplasia (e.g., other colorectal adenomas or CRC).[29–31] Moreover, certain well-characterized genetic risk factors for CRC (e.g., *APC* or *MSH2*/*MLH1* mutations) enable the identification of cohorts with an exceedingly high lifetime risk for CRC, which have already been shown to benefit from chemopreventive interventions.[32,33]

**Table 1.** Goals of CRC Chemoprevention

| |
|---|
| Short-term goals |
| Modulate validated mechanistic biomarkers |
| Regress prevalent preinvasive neoplastic lesions (i.e., adenomas) |
| Suppress recurrent preinvasive neoplastic lesions after definitive surgical resection |
| Prevent incident preinvasive neoplastic lesions |
| Long-term goals |
| Primary cancer prevention (i.e., general population at average risk for CRC) |
| Provide an alternative/complement to broad-based cancer screening |
| Delay initial colorectal cancer screening |
| Reduce risk of several chronic diseases (e.g., cancer and cardiovascular disease) by inhibiting or delaying their emergence |
| Secondary cancer prevention (i.e., screened cohorts at increased risk for CRC) |
| Improve efficacy of surveillance and its associated interventions |
| Address the effect of missed/complicated preinvasive neoplastic lesions |
| 10%–15% polyp miss rate |
| 5%–25% rate of flat adenomas |
| Preserve organs |
| Reduce intensity of surveillance by prolonging interexamination intervals |
| Improve safety and/or convenience of surveillance |
| Timing |
| Sedation |
| Complications |
| Reduce cancer risk in the colorectum or several organs at risk |
| Incident disease |
| Onset of disease |
| Tertiary cancer prevention (i.e., CRC survivors) |
| Reduce neoplasia recurrence |
| Palliate symptoms and improve quality of life |
| Reduce treatment-related toxicities |

Data from Umar et al.[69]

Cancer prevention has short- and long-term goals (Table 1). In the short term, chemoprevention trials strive to show the efficacy of a compound or to prioritize it among several promising agents by evaluating effects against mechanistic biomarkers or preinvasive neoplasia. Longer-term goals include the development of agents that complement broad-based cancer screening or improve surveillance-based management of patients with colorectal adenomas or CRC survivors. Common diseases such as carcinogenesis, atherogenesis, and neuronal degeneration may be modulated by aspirin, folate, vitamins, and physical activity, suggesting common underlying mechanisms. Because chemoprevention is typically a systemic, molecularly based approach to disease management, the most effective and accepted agents may

ultimately be ones that are shown to reduce the risk of more than 1 cancer or perhaps a host of chronic health conditions common to aging populations.[34]

## Agent Identification and Prioritization for Cancer Chemoprevention

The development of chemopreventive agents follows a standard algorithm for drug development, starting with mechanistically based drug screens, preclinical efficacy tests, toxicology assessments, and an orderly sequence of carefully designed clinical trials.[35,36] Before advancement into clinical trials, promising agents are identified and then prioritized on the basis of complementary lines of evidence. Prioritization criteria are broadly based on efficacy data arising from 3 different modes of inquiry—observational, in vivo models, and in vitro models—as outlined below.

### Observational Studies

Many candidate chemopreventive agents have come to attention on the basis of epidemiological data derived from their use for other conditions (e.g., oncologic, rheumatologic, endocrinologic, pulmonary, or gastroenterologic treatments). For example, lead compounds—including aspirin—have arisen from large observational studies, such as the American Cancer Society's Cancer Prevention Study II,[37,38] the Health Professionals Follow-up Study,[39] and the Nurses' Health Study.[39] These cohort studies collected questionnaires and, in some cases, biologic samples to assess exposure history and to document a broad range of outcomes, including colorectal adenoma and CRC incidence (e.g., Health Professionals Follow-up Study and Nurses' Health Study) and CRC-associated mortality (Cancer Prevention Study II). Important observational leads have also surfaced from secondary analyses of randomized controlled trials of interventions that were not specifically intended to reduce CRC. Selenium and conjugated equine estrogen, for example, recently gained attention as promising chemopreventive agents in the course of 2 large randomized, controlled trials evaluating other health effects.[40,41] Although positive epidemiological data are helpful, clinical studies of agents identified solely in this manner—particularly dietary compounds—have generally been disappointing in the prevention of CRC[42–44] and other cancers.[45,46] For this reason, agents are now prioritized on the basis of complementary lines of evidence, rather than any 1 type of data in isolation.

### In Vivo Efficacy Studies

A second strategy for identifying promising chemopreventive agents involves in vivo testing in animal models of carcinogenesis. The earliest carcinogen-based animal models used azoxymethane or dimethylhydrazine to induce ACF within 4–8 weeks or to induce adequate numbers of adenomas and carcinomas within 30–52 weeks (as reviewed elsewhere[47]). Investigators have since developed animals with germline defects in key regulatory genes including *APC*,[48–50] mismatch repair genes,[51–54] and SMAD,[55,56] among others. These animal models are an efficient way to probe molecular pathways underlying human germline disorders such as FAP or hereditary nonpolyposis CRC. In these disorders, advanced neoplasia tends to occur earlier than when disease is exogenously induced by carcinogen exposure.

Animal models have limitations, such as the tendency of APC-derived models to develop small-intestinal rather than colonic neoplasia. Even so, humans with germline APC defects often develop both types of neoplasia, much like APC-derived mice. Although their predictiveness for human carcinogenesis and its response to chemopreventive interventions is inexact, these animal models are quite useful. This is evident from the concordance between animal and human data on aspirin,[57–63] celecoxib,[33,64,65] and calcium.[66–68] As of 2002, more than 200 chemoprevention studies in animal models have been published, providing preliminary insights into a broad array of candidate agents.[22,47,69] To date, few of these compounds have been evaluated in human trials, which remain the rate-limiting factor in agent testing for CRC prevention.

### In Vitro Molecular Targeting

Insights into molecular mechanisms underlying neoplasia have led to the development of agents that target aberrations responsible for disease initiation and progression[69] (Table 2). The ideal chemopreventive target, if it exists, is unknown, but it likely relates to functional derangements that are unique to neoplasia and are differentially expressed in neoplastic vs. normal tissues.[5] In addition, the target should be pharmacologically accessible and responsive to modulation by the proposed agent. Finally, appealing targets are typically characterized by overexpression or overactivity, because molecular functions are more easily inhibited than replaced.

Agent prioritization also includes assessments of in vitro parameters such as intracellular concentrations, receptor binding, enzyme modulation, and intracellular signaling, depending on the agent's hypothesized activ-

**Table 2.** CRC Prevention Targets, Agents, and Associated Mechanisms

| Target | Class(es) | Examples | Possible mechanism(s) |
|---|---|---|---|
| Bile acids | Nutritional agent, synthetic bile acid | Calcium carbonate,[66] ursodiol | Inhibition of proliferation; reduction of cytotoxins; prevention of oxidative injury; modulation of protein kinase C; modulation of phospholipase $A_2$ expression |
| COX-1 and COX-2 | NSAID | Aspirin,[57,58] sulindac | COX-1 and COX-2 inhibition; inhibition of prostaglandins; inhibition of thromboxanes; reduction of proliferation; induction of apoptosis; antiangiogenesis |
| COX-2 | COXIB | Celecoxib,[33,169] rofecoxib[270] | COX-2 inhibition; inhibition of prostaglandins; inhibition of thromboxanes; reduction of proliferation; induction of apoptosis; antiangiogenesis |
| Cyclin-dependent kinase (CDK) | CDK inhibitors | Selenium[271] | Cell-cycle control; inhibition of cell growth |
| Epidermal growth factor receptor (EGFR) | EGFR inhibitors | EKB-569,[185,272] gefitinib (Iressa) | EGFR kinase inhibition; inhibition of mitogenic signal transduction/proliferation; induction of apoptosis; apoptosis induction in tumor cells; reversal of ras oncogenic activation |
| Farnesyl transferase | Farnesyl transferase inhibitors (FTI) | Dehydroepiandrosterone,[273] perillyl alcohol, FTI-276, batimastat[274] | |
| Matrix metalloproteinase (MMP-7) | MMP-7 inhibitor | Neovastat[275, 276] | Maintenance of basement membrane integrity |
| Ornithine decarboxylase (ODC) | ODC inhibitors | Eflornithine[188] | Polyamine synthesis blockade; reduction of proliferation |
| Peroxisomal proliferator–activated receptor (PPAR) gamma | PPAR ligands | GW7845,[277] BRL 49653[278] | Reduction of proliferation; regulation of inflammatory cytokines |
| DNA methylation | Methyl donors | Folate,[87] methionine, *S*-adenosyl methionine | Sustenance of purine and thymidine metabolism for DNA and RNA synthesis<br>Maintenance of methylation |
| Vascular endothelial growth factor (VEGF) | VEGF inhibitors | PTK787/ZK 222584[279] | Antiangiogenesis |
| Vitamin D receptor | Vitamin D | 1,25-dihydroxyvitamin D(3)[135] | Growth inhibition; promotion of cellular differentiation |

ity. Modulation of an in vitro subcellular target at agent concentrations achievable in vivo provides an important—but not necessarily sufficient—measure of chemopreventive potential. For example, an agent may show in vivo efficacy without modulating the proposed target in vitro because the compound works primarily by an alternative mechanism or depends on the tissue microenvironment for efficacy (or for other reasons altogether). Despite certain limitations, given the high cost of agent development, mechanistic data from in vitro studies help to prioritize agents for further research investment.

## Clinical Evaluation of Lead Compounds

The earliest in vivo study of a compound with chemopreventive potential against colorectal neoplasia dates to 1980, when carcinogen-exposed rats were treated with 5-fluorouracil (5-FU).[70] Although 5-FU has been a mainstay of CRC chemotherapy for more than 30 years, its preventive potential was largely unexplored. Initial trials of CRC patients randomized to adjuvant 5-FU vs. placebo generally failed to record adenoma recurrence, thus missing an opportunity to validate the chemopreventive efficacy of this drug. In addition, chemotherapeutic development of 5-FU tested relatively brief exposures to high doses in persons with CRC; therefore, the chemopreventive potential of chronic exposure to lower doses in persons at risk for CRC has yet to be systematically studied.

Case reports of FAP patients treated with 5-FU for chemoprevention or as a part of a multiagent chemotherapeutic regimen have described significant reductions in colorectal adenoma burdens.[71,72] Moreover, significant anti-IEN activity has been observed in nontargeted organs as well. For example, clinicians treating colon cancer patients with systemic 5-FU incidentally observed regression of actinic keratosis, a pre-malignant skin condition.[73] Clinical testing subsequently confirmed efficacy against actinic keratosis, and a topical formulation of 5-FU gained Food and Drug Administration approval for this indication in 1970. This illustrates how evidence of efficacy against extracolonic IEN in the context of CRC treatment might translate into other chemopreventive applications. Prospective assessment of ACF or adenoma

**Table 3.** Colorectal Neoplasia Prevention Trials Evaluating Dietary Fat and Fiber

| References | Intervention regimen | Sample size[a] | Design/cohort[b] | Primary results |
|---|---|---|---|---|
| DeCosse[280] | Wheat bran fiber (WBF) 2.2 g/day + placebo vs. WBF 2.2 g/day) + vitamin C 4 g/day + vitamin E 400 mg/day vs. WBF 22.5 g/day + vitamins C + E × 48 mo | 58 | DBRCT in FAP patients | Rectal adenoma number—nonsignificant reduction with high-dose fiber + vitamins |
| MacLennan[281] | Low fat intake (<25% of total calories) vs. wheat bran 25 g/day vs. beta-carotene 20 mg/day—7-arm factorial trial × 24–48 mo | 424 | Factorial, partially DBRCT in patients with prior adenoma | Adenoma incidence—no overall effect; large adenoma (≥1 cm) incidence—reduction with WBF + low-fat combination diet[c] |
| Earnest,[283] Alberts[43] | WBF 2 g/day vs. WBF 13.5 g/day × 36 mo | 1429 | DBRCT in patients aged 40–80 yr with prior adenoma (≥3 mm) | Adenoma incidence—1% reduction (NS); persons with adenomas—12% reduction (NS) |
| Schatzkin[44] | Low fat intake (≤20% of total calories) + fiber 18 g/1000 kcal + fruits and vegetables (5–8 servings/day) vs. typical US diet × 48 mo | 2079 | PBRCT in patients aged ≥35 yr with prior adenoma | Adenoma incidence—no effect (RR, 1.00) |
| Faivre[284] | Ispaghula husk 3.8 g/day vs. calcium 2 g/day vs. placebo | 655 | DBRCT in patients aged 35–75 yr with prior adenoma | Adenoma incidence—34% reduction with calcium (NS); 67% increase[c] with fiber |

NS, not significant.
[a]Number randomized.
[b]Trial designs; PBRCT, partially blind, randomized, controlled trial; DBRCT, double-blind, randomized, controlled trial.
[c]Statistically significant ($P < 0.05$).

recurrence rates among cancer patients poses certain technical challenges, but this strategy might expose the preventive or promotional sequelae of adjuvant chemotherapy. Finally, the inclusion of endoscopic assessments of colorectal neoplasia within trials of agents targeting other tissues might provide opportunities to streamline at least 1 part of the agent development process.

## Dietary Agents

The first clinical chemoprevention study in CRC involved the administration of ascorbic acid (vitamin C) vs. placebo to a small group of patients with FAP.[74] Although the intervention was clinically unimpressive, the study's innovative design made it a prototype for future trials. Over the following 2 decades, more than 20 primary[41,75] and secondary[42–44,66] prevention trials have tested the efficacy of promising agents and, in so doing, further refined the design of cancer prevention trials (Tables 3–5).

To date, trials of dietary agents in colorectal neoplasia prevention have shown limited effects with broad-based (e.g., increases in fiber, fruits, and vegetables and a lower fat intake), intermediate (e.g., supplementation with various whole foods, such as wheat bran or cruciferous vegetables), or specific (e.g., supplementation with 1 or more vitamins or mineral components) nutritional modifications. Nonetheless, several lines of evidence support a role for dietary modifications in the prevention of colorectal neoplasia. Early cross-cultural and ecological comparisons suggested strong correlations between fat intake and CRC incidence.[76,77] Furthermore, a large number of observational studies suggested that vegetables, fruits, whole grains, dietary fiber, moderate alcohol intake, tea compounds, and certain micronutrients might be protective against CRC and that excess alcohol intake and certain fatty acids might increase CRC risk.[78,79] Several animal studies confirmed that dietary changes might reduce cancer risk.[47,80,81] The sections that follow summarize mechanistic, observational, and experimental data on promising chemopreventive compounds derived from dietary sources that have advanced into higher-phase clinical testing.

### Antioxidants

Most fruits and vegetables contain antioxidant vitamins and minerals. Representative examples such as carotenoids (vitamin A precursors), retinoids (vitamin A), ascorbic acid, α-tocopherol (vitamin E), and selenium can neutralize free radicals, thus reducing intrinsic oxidative and carcinogen-induced DNA damage.[82] In addition, certain studies suggest that antioxidants may inhibit tumorigenesis by stimulating the immune system.[83]

Observational data on the ability of antioxidants to reduce colorectal neoplasia are difficult to tease apart

**Table 4.** Colorectal Neoplasia Prevention Trials Evaluating Vitamins, Calcium, or Selenium Supplements

| Reference | Intervention regimen | Sample size[a] | Design/cohort[b] | Primary results |
|---|---|---|---|---|
| Duris[285] | Calcium (NOS) × mean 37 mo | 175 | Case series of patients with prior resected adenoma or CRC | Adenoma recurrence—76% reduction; cumulative survival in cancer patients after resection—improved |
| Hofstad[98,286] | Calcium 1.6 g + beta carotene 15 mg + vitamin C 150 mg + vitamin E 75 mg + selenium 101 μg/day vs. placebo × 36 mo | 116 | DBRCT in patients with small (<1 cm), retained adenomas | Growth of small adenomas—no effect; adenoma incidence/recurrence—increased[c]; fecal bile acids—no effect |
| Baron[66] | Calcium 3.0 g/day vs. placebo × 48 mo | 930 | DBRCT in patients with prior adenoma | Patients with adenoma incidence—19% reduction[c]; adenoma number—24% reduction[c]; adenoma incidence—34% reduction (NS) |
| Faivre,[284] Bonithon-Kopp[129] | Calcium 2.0 g/day vs. 3.8 g/day ispaghula husk vs. placebo × 36 mo | 655 | DBRCT in patients with prior adenoma; 35–75 yr old | |
| Clark[40] | Selenium 200 μg/day vs. placebo × 6.4 yr | 1312 | DBRCT of skin cancer subjects | CRC incidence—58% reduction[c] |
| Greenberg,[42] Baron[101] | Beta-carotene 25 mg/day vs. vitamin C 1 g/day + vitamin E 400 μg/day vs. both vs. placebo × 4 yr | 864 | 2 × 2 factorial DBRCT in patients with prior adenomas | Adenoma incidence: beta-carotene, 1% increase (NS); vitamin C, 8% increase (NS) |
| Malila,[99] Albanes[100] | Alpha-tocopherol 50 mg/day, beta-carotene 20 mg/day, both, or placebo | 29,133 (cancer analyses); 15,538 (adenoma analyses) | DBRCT of Finnish male smokers 50–69 yr old | Alpha-tocopherol: adenoma incidence, 66% increase[c]; CRC incidence, 21% reduction (NS); beta-carotene: adenoma incidence, 2% reduction (NS); CRC incidence, 5% increase (NS) |

NOS, not otherwise specified; DBRCT, double-blind, randomized, controlled trial; NS, not significant.
[a]Number randomized.
[b]Trial designs.
[c]Statistically significant result ($P < 0.05$).

from the effects of bioactive agents admixed in the same food products.[84] Both retrospective[85–89] and prospective studies of antioxidant vitamins have shown equivocal effects against colorectal neoplasia. Selenium is more convincingly associated with preventive effects in ecological studies of crop selenium content,[90] but correlations between serum or toenail selenium concentrations and reductions in colorectal neoplasia have been inconsistent.[91–94]

In terms of clinical studies, 2 phase II trials evaluating antioxidant combinations reported reductions in colorectal adenoma proliferation and recurrence,[95,96] but 4 larger trials (Table 4) conducted over longer durations failed to confirm preventive efficacy against adenoma or cancer incidence.[42,97–99] The largest of these trials involved more than 15,000 Finnish male smokers who were administered α-tocopherol, beta-carotene, or both for several years. Surprisingly, this trial reported a statistically significant 66% increase in colorectal adenomas,[99] despite a statistically nonsignificant 16% reduction in CRC incidence.[100] These perplexing data do not support broad-scale use of antioxidants for cancer prevention. More recently, a subgroup analysis from the Dartmouth trial focusing on beta-carotene in subjects who neither smoked cigarettes nor drank alcohol suggested a 44% reduction in recurrent adenomas.[101] By contrast, beta-carotene increased the risk of adenoma recurrence among participants who used tobacco or alcohol. These positive results are provocative and await confirmation in other trials, including an ongoing European trial involving 15,000 randomized subjects. This Supplementation en Vitamines et Mineraux Antioxydants study will evaluate whether a combination of ascorbic acid, vitamin E, beta-carotene, selenium, and zinc taken for 2 years can reduce the incidence of several diseases, including CRC.[102] Preliminary data have already reported significant changes in serum concentrations of the study vitamins.[103]

Clark et al.[40] tested the chemopreventive effect of selenium supplementation as a secondary end point in a placebo-controlled, randomized trial of 1312 persons with a history of skin cancer. Although few participants developed CRC after more than 6 years of follow-up, a statistically significant 58% reduction in CRC incidence occurred among individuals randomized to selenium supplementation. Although far from definitive, these

**Table 5.** Colorectal Neoplasia Prevention Trials Evaluating Drugs with Chemopreventive Potential

| Reference | Intervention regimen | Sample size[a] | Design/cohort[b] | Primary results |
|---|---|---|---|---|
| Larson/McLeod (unpublished)[c] | Ursodiol (NOS) vs. placebo × 1 yr | 716 | DBRCT in patients with prior adenoma or early-stage CRC | Adenoma incidence—43% reduction (NS); large adenoma (>1 cm) incidence—50% reduction (NS); adenoma size—77% reduction (NS) |
| Alberts[287] | Ursodiol 8–10 $mg \cdot kg^{-1} \cdot day^{-1}$ vs. placebo × 3 yr | 1285 | DBRCT in patients with prior adenoma | Adenoma incidence—7% reduction (NS); advanced adenoma incidence—14% reduction (NS) |
| Rossouw[41] | Conjugated equine estrogen 0.625 mg/day + medroxyprogesterone acetate 2.5 mg/day vs. placebo × 5.2 yr | 16,608 | DBRCT in healthy women 50–79 yr of age | CRC incidence—37% reduction[b] |
| Gann[75] | Aspirin 325 mg/day vs. placebo × 5 yr | 22,071 | DBRCT in healthy physicians 40–84 yr old | Advanced adenoma incidence—14% increase (NS); CRC incidence—15% reduction (NS) |
| Baron[57] | Aspirin 81 vs. 325 mg/day vs. placebo × 3 yr | 1211 | DBRCT in patients with prior adenoma | 81 mg/day: adenoma incidence, 19% reduction,[d] advanced adenoma incidence, 41% reduction[d]<br>325 mg/day: adenoma incidence, 4% reduction (NS); advanced adenoma incidence, 17% reduction (NS) |
| Benamouzig[155e] | Aspirin 160–300 mg/day vs. placebo × 4 yr | 291 | DBRCT in patients with prior adenomas | Persons with adenomas—27% reduction (borderline significance; $P = 0.08$); adenoma >1 cm in diameter—83% reduction[d] |
| Sandler[158] | Aspirin 325 mg/day vs. placebo × 3 yr | 635 | DBRCT in patients with prior resected early-stage CRC | Adenoma incidence—35% reduction[d] |

NOS, not otherwise specified; DBRCT, double-blind, randomized, controlled trial; NS, not significant.
[a]Number randomized.
[b]Trial designs.
[c]Trial by Larson and McLeod (November 2000 interim analysis on 258 of 716 patients published on the AXCAN Pharmaceuticals Web site; http://www.axcan.com/pressdetails.aspx?lang=en-ca&m=61&n=2&id=2000).
[d]Statistically significant ($P < 0.05$).
[e]Interim result at 1 yr; intended duration of intervention is 4 yr.

data have prompted other inquiries into the chemopreventive potential of selenium. For example, the Selenium and Vitamin E Cancer Prevention Trial is assessing the effects of selenium with or without vitamin E against incident prostate cancer in men older than 50 years. The effects of selenium against colorectal neoplasia will be determined through colonoscopic evaluation of a subset of these participants. In addition, a large, ongoing phase III trial at the University of Arizona is examining the efficacy of selenium with or without celecoxib in reducing recurrent colorectal adenomas.

### Folate and Methionine

Fresh fruits and leafy green vegetables are rich in folate, whereas red meat, chicken, and fish have relatively high concentrations of methionine. Folate and methionine both supply methyl groups necessary for DNA synthesis and gene expression. Therefore, diets deficient in folate or methionine may contribute to colorectal carcinogenesis by impairing DNA synthesis, repair, or transcriptional expression.[104] Case-control and prospective studies suggest that dietary folate and methionine exert chemopreventive effects against colorectal carcinogenesis.[105–110] The amount,[47] duration,[95] and timing of intake may influence the degree of protection that these dietary components confer. Indeed, participants with the highest levels of folate intake in the Health Professionals Follow-up Study and the Nurses' Health Study had an approximately 25%–35% lower risk for distal colorectal adenomas, and women who took folate-rich multivitamins for more than 14 years had a 75% risk reduction as compared with nonusers. Dietary and genetic factors may modulate the proposed chemopreventive effects of folate and methionine. Certain studies have shown that heavy alcohol consumption and polymorphisms in methylenetetrahydrofolate reductase may reduce the availability of methyl groups, thus altering the chemopreventive effects of folate or methionine.[39,111–115]

Folate status may be an important determinant of neoplastic risk in persons with ulcerative colitis (UC).

Lashner[116] reported an inverse association between red blood cell folate concentrations and colorectal dysplasia and also observed a reduced risk of colorectal dysplasia among patients who took folate supplements.[117,118] Three phase II trials have associated short-term folate supplementation with improved DNA methylation patterns and strand integrity, as well as reductions in mucosal proliferation.[119–121] Currently, at least 2 phase III trials are evaluating the efficacy of folate supplements vs. placebo in preventing colorectal adenomas in patients with prior sporadic neoplasia.

### Calcium Carbonate and Vitamin D

Secondary bile acids may play an important role in colorectal carcinogenesis.[122,123] Calcium binds bile and fatty acids in the form of insoluble soaps, which effectively sequesters these mutagenic substances from harmful contact with epithelial cells. In addition, calcium may directly inhibit epithelial proliferation within the colorectum by modulating protein kinase C activity, stabilizing membranes, or modifying K-*ras* mutations.[124]

Case-control and prospective epidemiological studies show moderate and fairly consistent inverse associations between calcium intake and CRC risk[125]; however, phase II trials have yielded somewhat inconsistent data. For example, Pence[126] reported significant reductions in colonic epithelial proliferation after calcium supplementation, whereas several larger trials failed to confirm this observation.[66,127] Randomized, placebo-controlled phase III trials have shown that calcium reduces adenoma recurrence in cohorts at increased risk for CRC (Table 4). Most recently, Baron et al.[66,128] reported a statistically significant 19% reduction in patients with recurrent adenomas and a 44% reduction in advanced adenomas among individuals taking a calcium carbonate supplement. It is interesting to note that chemopreventive effects were observed as early as 1 year after treatment was initiated, suggesting that calcium acts relatively quickly.[66] These promising results were supported by another multicenter, randomized, placebo-controlled trial of 655 patients with prior adenomas, among whom those treated with calcium (and/or fiber) supplements had a nonsignificant 34% reduction in recurrent adenomas.[129] Collectively, these 2 trials suggest that calcium supplements are well tolerated and achieve a statistically significant, albeit modest, reduction in recurrent adenomas. The US Women's Health Initiative is evaluating approximately 45,000 postmenopausal women who have been randomized to take calcium and vitamin D vs. placebo for a mean of 9 years. This trial will evaluate preventive effects against several common diseases of aging, including CRC and osteoporosis.[130]

The chemopreventive properties of vitamin D may relate to its ability to modulate both calcium absorption and gene expression. Geographic studies provided the earliest suggestion of a link between CRC risk and vitamin D concentrations.[131] These observational data were corroborated by mechanistic inquiries showing that vitamin D may prevent CRC by inducing E-cadherin, by inhibiting β-catenin,[132] or by other anti-proliferative effects.[133,134] A large epidemiological study of the American Cancer Society cohort reported a 29% reduction in CRC risk among individuals with the highest vitamin D intakes from dietary or supplemental sources.[135] Phase III trials testing the efficacy of vitamin D for the prevention of CRC have been planned.

### Dietary Fiber

Fiber is crudely defined as the dietary fraction that is resistant to human digestion and absorption. Fiber may increase stool bulk and stimulate intestinal transit, thereby reducing epithelial exposure to intraluminal carcinogens. Dietary fiber also reduces procarcinogenic secondary bile acids and increases the concentration of short-chain fatty acids. Despite evidence that supplementation provides other health benefits (e.g., reduced cardiovascular disease and improved glycemic control), fiber seems to have little or no consistent benefit in reducing colorectal adenoma risk. Many observational studies, as well as phase II and III trials, have failed to associate cancer-preventive effects with dietary fiber supplementation[43,44,129] (Table 3). Indeed, in 1 phase III trial, fiber supplementation increased participants' risk for recurrent adenomas by 67%.[129] By contrast, the European Prospective Investigation into Cancer and Nutrition study described a significant 25% risk reduction in CRC among individuals who consumed the highest vs. lowest quintile of dietary fiber.[136] Trials involving a different type of fiber administered in a different manner (e.g., earlier, later, or according to yet-to-be-determined regimens), to other cohorts (e.g., younger persons or those without prior adenomas), or using other efficacy measures (e.g., cancer incidence or cancer-associated mortality) may enhance our understanding of fiber's chemopreventive potential.[137] At least 2 ongoing international trials are evaluating the efficacy of resistant starch coadministered with aspirin in patients at hereditary risk for CRC[138] (Table 6).

## Pharmaceutical Agents

The most promising chemopreventive agents are effective, well tolerated, and affordable. Although most

**Table 6.** Ongoing Colorectal Neoplasia Prevention Trials

| Investigator/lead institution | Cohort (sample size) | Agents | Control | Primary goal | Phase |
|---|---|---|---|---|---|
| Bresalier/M. D. Anderson Cancer Center | History of sporadic adenoma or CRC (180) | Aspirin, sulindac, ursodiol | Placebo | Aberrant crypt foci (ACF) regression | II |
| Kirsch/National Cancer Institute | Patients with sporadic rectal ACF (40) | Celecoxib | Placebo | ACF regression and prevention | II |
| Larson/MacLeod/ AXCAN | History of sporadic adenoma or CRC (594) | Ursodiol | Placebo | Adenoma prevention | II |
| Lynch/M. D. Anderson Cancer Center | Phenotypic adults with FAP (152) | Celecoxib + eflornithine | Placebo | Adenoma regression | II |
| Lynch/M. D. Anderson Cancer Center | Prephenotypic children with FAP (N/A) | Celecoxib | Placebo | Adenoma suppression | II |
| Lynch/M. D. Anderson Cancer Center | HNPCC patients or gene carriers (83) | Celecoxib | Placebo | Mucosal biomarker modulation | II |
| Shiff/Rockefeller University | Average or above-average risk of CRC (130) | Sulindac and curcumin | None | Mucosal biomarker modulation | II |
| Alberts/Arizona Cancer Center | History of sporadic adenoma (1200) | Celecoxib +/− selenium | Placebo | Adenoma prevention | III |
| Baron/Norris Cotton Cancer Center | History of sporadic adenoma (1800) | Aspirin +/− folate | Placebo | Adenoma prevention | III |
| Benamouzig/APA CC Study Group | History of sporadic adenoma, aged 18–75 yr, (274) | Aspirin | Placebo | Adenoma prevention | III |
| Berkel/Hipple Cancer Center | History of sporadic adenoma (980) | Piroxicam +/− calcium | Placebo | Adenoma prevention | III |
| Bertagnolli/Strang Cancer Prevention Center | History of sporadic adenoma (1000) | Celecoxib | Placebo | Adenoma prevention | III |
| CAPP-1 | FAP carriers (400) | Aspirin + resistant starch | Placebo | Adenoma prevention | III |
| CAPP-2 | HNPCC gene carriers (1000) | Aspirin + resistant starch | Placebo | Adenoma/cancer prevention | III |
| Giovannucci/Brigham & Women's Hospital | History of sporadic adenoma (1000) | Folate | Placebo | Adenoma prevention | III |
| Merck | History of sporadic adenoma (2400) | Rofecoxib | Placebo | Adenoma prevention | III |
| Meyskens/University of California Irvine | History of sporadic adenoma (240) | Sulindac + eflornithine | Placebo | Adenoma prevention | III |
| SUVIMAX | French volunteers aged 35–60 yr (12,749) | Vitamin C, vitamin E, beta-carotene, selenium, +/− zinc | Placebo | CRC prevention, mortality reduction | III |
| UK-CAP | History of sporadic adenoma (1000) | Aspirin +/− folate | Placebo | Adenoma prevention | III |
| Women's Health Initiative | Postmenopausal women aged 50–79 yr | Low-fat diet vs. calcium + vitamin D vs. hormone-replacement therapy | Placebo | CRC incidence | III |
| Women's Health Study/Harvard | Healthy female health professionals aged ≥45 yr (39,876) | Aspirin +/− vitamin E | Placebo | CRC incidence | III |

NOTE. Ongoing prevention trials have been extracted from the National Cancer Institute's Computer Retrieval of Information on Scientific Projects (http://crisp.cit.nih.gov/) and clinical trials Web sites (http://cancer.gov/clinical_trials) or published literature.
CAPP, concerted action polyp prevention; HNPCC, hereditary non polyposis colorectal cancer; N/A, not available; SUVIMAX, Supplementation en Vitamines et Mineraux Antioxydants; UK-CAP, United Kingdom Colorectal Adenoma Prevention Trial.

agents fall short of this ideal in some regard, they may still prove useful. For example, people at average or slightly increased risk for CRC may not abide frequent, severe, or prolonged toxicities. By contrast, high-risk individuals, such as those with FAP, hereditary nonpolyposis CRC, UC with dysplasia, or a strong family history of CRC may tolerate moderate toxicities without great concern if an agent has profound preventive activity. More than 50 pharmacologic agents, singly or in combination, have been evaluated as chemopreventive agents against colorectal carcinogenesis in preclinical models.[22,69] Examples of agents tested in clinical trials or contemplated for translation into clinical trials are described in the following sections.

### Nonsteroidal Anti-inflammatory Drugs, Nonsteroidal Anti-inflammatory Drug Derivatives, and Cyclooxygenase-2 Inhibitors

Among the agents currently under investigation for the prevention of CRC, NSAIDs are the most compelling in terms of the volume, variety, and consistency of preliminary data. Indeed, NSAIDs have been shown to exert effects against all clinical stages of colorectal neoplasia (e.g., ACF, adenomas, cancer, and cancer-associated mortality). The chemopreventive mechanisms of NSAIDs were first explored in the early 1970s, yet we still do not understand them completely.[24] The best-described activity of NSAIDs relates to the inhibition of 1 or both COX enzymes (COX-1 and COX-2). COX catalyzes the conversion of arachidonic acid into bioactive eicosanoids, including prostaglandins and lipoxygenases. COX-1 is constitutively expressed in a wide range of tissues, whereas COX-2 is constitutively expressed in only a few. A variety of inflammatory and neoplastic stimuli (e.g., cytokines and mutagens) induce massive COX-2 overexpression, which is a common feature of colorectal preinvasive and invasive neoplasia.[139] COX-2 is overexpressed and seems to be pathogenically important in extracolonic neoplasias as well.[139] For example, recent investigations have correlated COX-2 overexpression with poorer survival among patients with CRC[140] and other cancers.[141–144] By inhibiting 1 or both COX enzymes, NSAIDs may reduce proliferation, induce apoptosis, promote immunologic surveillance, or reduce neoangiogenesis.[145]

In addition to the aforementioned COX-dependent mechanisms, NSAIDs may also induce COX-independent anticancer effects by stimulating peroxisome proliferator–activated receptor (PPAR) ligands[146] or by blocking phosphorylation of Akt.[147] The case for COX-independent effects was strengthened when the anticancer effects of COX-2 selective COXIBs were described in a COX-2–null cell line.[148] Nevertheless, most of these activities occur at in vitro concentrations far higher than those achievable in humans (up to 1–5 μmol/L); therefore, their clinical relevance is uncertain.[149]

Regardless of which mechanisms specifically account for the efficacy of NSAIDs against CRC, substantial and consistent effects have been shown in more than 90 of the ≥100 rodent studies published to date. Complementing data from carcinogen-induced and genetically induced CRC animal models, more than 30 epidemiological studies confirm 40%–50% reductions in colorectal adenomas, CRCs, and cancer-associated mortality among users of aspirin or other NSAIDs, as compared with nonusers.[24] These risk reductions are consistently observed across study designs, agents (i.e., aspirin and several other NSAIDs), and cohorts, regardless of potential confounders, such as age, sex, ethnicity, and personal or familial risk factors.

More than 15 case series and at least 4 randomized, controlled trials have proven the efficacy of NSAIDs (primarily sulindac) in reducing the adenoma burden of persons with FAP,[32] presumably through regression of prevalent adenomas in this high-risk cohort. In a recent case series, this effect was sustained over a mean of 63.4 months.[150] By contrast, in a recent trial of prephenotypic FAP patients, sulindac did not significantly reduce or delay the emergence of colorectal adenomas.[27] A recent trial of aspirin with or without resistant starch vs. placebo confirmed these findings, showing no significant reduction in incident colorectal adenomas among prephenotypic patients harboring *APC* mutations.[151]

The effects of NSAIDs against sporadic adenomas in phase II trials are mixed but encouraging.[152–154] For example, 2 small trials[153,154] showed no significant adenoma regression when sulindac was administered at standard doses for a period of months, but a third trial[152] showed significant regression. Four phase III trials of aspirin have been published to date (Table 5). The Physicians' Health Study, the only large, randomized, placebo-controlled trial of aspirin vs. placebo in healthy individuals, reported no significant reduction in CRC incidence (relative risk [RR], 1.15; 95% confidence interval [CI], 0.80–1.65) or in situ cancers or polyps (RR, 0.86; 95% CI, 0.68–1.10).[75] By contrast, 3 recent placebo-controlled trials involving patients at greater than average risk for CRC (because of prior adenomas or cancer) showed significant reductions in recurrent adenomas among those treated with aspirin for ≥1 year.[57,58,155] Sandler et al.[58] randomized 635 CRC survi-

vors to 325 mg/day of aspirin vs. placebo. After a median follow-up of 12.8 months, the study showed a significant reduction in the number of patients with incident adenomas (17% vs. 27%; $P = 0.004$) and a significant delay in the time to a first adenoma ($P = 0.022$) in the group that was randomized to aspirin. The second study, by Baron et al.,[57] randomized 1121 patients with prior colorectal adenomas to aspirin 81 or 325 mg/day vs. placebo and reported 19% and 4% reductions, respectively, in the number of people with 1 or more adenomas. Effects against advanced adenomas were more striking, with 41% and 17% reductions in the groups taking 81 or 325 mg/day vs. placebo, respectively. The third phase III trial showed interim preventive benefits of aspirin in 272 patients with prior adenomas after only 1 year of administration, but this trial is still ongoing.[155]

In aggregate, these trials confirm that typical doses of aspirin administered for a relatively brief period reduce colorectal neoplasia in persons at moderately high risk for CRC. The inverse dose response observed in the Baron trial and the absence of effects in persons at average risk are somewhat baffling, but these questions and many others may be resolved by 2 large, ongoing trials (Table 6). In the United Kingdom Colorectal Adenoma Prevention Trial, 894 patients with prior adenomas are receiving 300 mg of aspirin vs. placebo over 3 years.[156] The second trial, the Women's Health Study, involves 39,876 female health professionals randomized to 100 mg of aspirin every other day vs. placebo for 8 years.[157]

The chemopreventive efficacy of nonselective NSAIDs has been reasonably established, as has the toxicity profile. The risk for NSAID-related ulcers and associated complications has been shown to increase significantly with older age, certain concomitant medications, a history of ulceration, and serious comorbid conditions. Indeed, as many as 1%–2% of chronic aspirin users experience gastroduodenal ulceration or bleeding.[158] Consequently, these factors must be taken into account when considering this class of agents for chemopreventive indications.[159–161]

The therapeutic index (TI) of NSAIDs for cancer chemoprevention might be improved by using any of several strategies. One approach involves specific targeting of key determinants of colorectal carcinogenesis, such as COX-2. COX-2 is overexpressed in approximately 50% of colorectal adenomas and in 80%–85% of adenocarcinomas.[162] Increasing evidence from different sources suggests that COX-2 overexpression plays a pathogenic role in neoplastic progression. First, Oshima et al.[163] showed that Min mice lacking a functional *COX-2* gene had dramatically fewer adenomas. More recently, Sheng et al.[164] showed substantial reductions in cellular proliferation after treatment with a COX-2 selective COXIB, an effect that was reversed by prostaglandin $E_2$.[165] In addition, colonic cells that overexpress COX-2 are resistant to apoptosis.[166] Finally, overexpression of COX-2 was recently shown to induce mammary[167] and skin[168] carcinogenesis in 2 animal models. These data strongly implicate COX-2 in carcinogenesis and provide a compelling rationale for mechanistically targeting COX-2 for cancer prevention.

One phase II trial of celecoxib conducted in 83 patients with FAP showed the potential of COXIBs for chemoprevention. This randomized, controlled trial showed a mean 28% reduction (vs. 5% in the placebo arm; $P = 0.003$) in the number of colorectal adenomas among patients who were administered 400 mg of celecoxib twice a day over 6 months.[33] Significant reductions in the duodenal adenoma burden were also reported.[169] On the basis of the results of this small trial, the US Food and Drug Administration approved celecoxib in 1999 as an oral adjunct to the standard of care (e.g., endoscopic surveillance and surgery) for adults with FAP under the subpart H guidelines.

The durability of regressions achieved after 6 months of COX-2 inhibition has yet to be established, and the long-term consequences of this treatment in FAP are still unknown. Furthermore, direct correlations between reduced adenoma burden and definitive clinical outcomes (e.g., reductions in CRC morbidity and mortality or changes in surveillance practices) have yet to be proven. To address these and other uncertainties, trials are evaluating celecoxib in prephenotypic FAP patients, as well as its efficacy against prevalent adenomas when combined with another chemopreventive agent (eflornithine; see below) in adults with FAP (Table 6). These trials should provide important insights into the longer-term risks and benefits of celecoxib, as well as its potential use in combination regimens for high-risk patients. Data on the preventive potential of COXIBs in persons at risk for sporadic CRC will come from 4 ongoing trials conducted in patients with prior adenomas (3 testing celecoxib and 1 testing rofecoxib).

Sulindac sulfone, a derivative of sulindac with less gastroduodenal toxicity than the parent compound, was shown to reduce cancer development in carcinogen-treated rats,[170] but not in genetically predisposed mice.[171] One small phase II clinical trial showed stabilization of adenoma burden in patients with FAP treated for several months with sulindac sulfone.[172] The results of more definitive, longer-term trials have yet to be reported.

*R*-Flurbiprofen is an enantiomer of the nonselective NSAID flurbiprofen and is thought to have a lower propensity for upper gastrointestinal side effects than the parent compound. Preliminary studies in Min mice suggested significant reductions in intestinal neoplasia after treatment with *R*-flurbiprofen; however, human data have yet to be reported.[171,173] Similarly, nitric oxide–releasing NSAIDs were developed to improve the TI of traditional NSAIDs.[174] Although there are few data on preventive effects against CRC, this class of agents is conceptually appealing. Kashfi et al.[175] reported greater in vitro potency (e.g., antiproliferative, proapoptotic, and morphological effects) in certain cancer cell lines treated with nitric oxide–releasing NSAIDs as compared with traditional NSAIDs. In vivo data have yet to be published.

The role of COX-2 in colorectal tumorigenesis has recently been challenged, and COX-1 has been proposed as a reasonable target for cancer chemoprevention. Genetic disruption of *COX-1* has been shown to significantly reduce intestinal tumorigenesis,[176] and mofezolac, a COX-1 selective COXIB, was reported to reduce intestinal carcinogenesis in carcinogen-induced and genetically induced rodent models of colorectal neoplasia.[177] It is interesting to note that Wallace et al.[178] recently showed that concomitant inhibition of COX-1 and COX-2 induced gastric damage in rats, whereas inhibition of only 1 isoform did not. In aggregate, these data suggest that either COX-1 or COX-2 selective COXIBs may improve the TI of NSAIDs for cancer prevention.

Finally, combining NSAIDs or COXIBs with other chemopreventive agents may further improve the TI of COX inhibition, either by boosting NSAID/COXIB efficacy or reducing toxicities. Preclinical data confirm enhanced chemopreventive effects without enhanced toxicities when NSAIDs are coadministered with other agents (e.g., interleukin-12[179] and eflornithine[180–184]). In a recent study, Torrance et al.[185] dramatically reduced intestinal tumors in Min mice by using a combination of COX and epidermal growth factor signaling inhibitors. It is important to note that this effect was achieved even when sulindac was dose-reduced by 75%; this suggests intriguing opportunities for chronic administration of low-dose NSAIDs with reduced potential for untoward side effects. These preliminary data were recently confirmed and extended by Tortora et al.,[186] who showed marked cooperative effects against cancer cell lines through combined blockade of COX-2, epidermal growth factor receptor (EGFR) (Iressa; AstraZeneca, Wilmington, DE), and/or an antisense inhibitor of protein kinase A type I.

### Eflornithine

Eflornithine (also known as difluoromethylornithine) irreversibly inhibits ornithine decarboxylase, the rate-limiting step in polyamine synthesis.[187] Polyamines are ubiquitous in nearly all biological systems and have been implicated as promoters of cell proliferation and neoplasia. In colorectal carcinogenesis, the loss of functional APC is commonly associated with the overexpression of c-*myc*, which leads to increased ornithine decarboxylase RNA expression, polyamine synthesis, and epithelial proliferation.[188,189] Ornithine decarboxylase activity and polyamine content are significantly higher in colorectal adenomas and CRCs as compared with adjacent healthy tissues.[190–193] Eflornithine profoundly inhibits polyamine synthesis and tumorigenesis in carcinogen-driven, genetic, and xenograft-based animal models of colorectal carcinogenesis.[180,194–196] In addition, eflornithine has been shown to reduce the incidence, multiplicity, growth rate, and/or volume of ACF, adenomas, and carcinomas, suggesting activity against events that occur in the post-initiation phase of tumorigenesis.[181,197–200]

Originally developed as a cancer chemotherapeutic agent in the 1970s, eflornithine was promptly abandoned after phase II studies exposed significant toxicities (e.g., thrombocytopenia, nausea, vomiting, abdominal pain, diarrhea, and reversible hearing loss) in the 1980s.[201,202] Although dose-limiting toxicity terminated its development for chemotherapeutic indications, eflornithine was shown to have spectacular efficacy against infections with *Trypanosoma brucei gambiense* and *rhodesiense*, protozoans that cause African sleeping sickness. Efficacy against *Pneumocystis carinii* pneumonia also led to its use as a salvage drug for unresponsive disease. Eflornithine has subsequently undergone development as a chemopreventive agent with use of lower doses and different modes of delivery from those used for chemotherapy. In addition, sensitive measures have been implemented to identify and categorize toxicities that might be attributable to low doses administered over long durations.[203] Thus far, chemoprevention trials have shown suppression of ornithine decarboxylase activity and polyamine concentrations in relevant target tissues at doses of 0.2–1.0 $g \cdot m^{-2} \cdot day^{-1}$.[204–206] In patients with personal or family histories of colorectal neoplasia, eflornithine at doses as low as 0.2–0.4 $g \cdot m^{-2} \cdot day^{-1}$ over 12 months suppresses rectal polyamine levels.[207,208]

Additive and synergistic efficacy have been shown when eflornithine is coadministered with other chemopreventive agents in a variety of settings.[180–184,209–213] Five preclinical studies showed that eflornithine in combination with an NSAID such as piroxicam or aspirin

synergistically reduced intestinal neoplasia as compared with placebo, even when drug doses were reduced by as much as 50%.[180–184] This effect is being explored in 2 trials involving cohorts with a history of sporadic adenomas or FAP (Table 6). Synergistic efficacy has also been shown when eflornithine is combined with mitomycin C,[209] cyclosporine,[211] or selenium.[210] Because of its activity against carcinogenesis in a wide variety of organs, eflornithine may hold particular promise for individuals at risk for cancer at multiple sites, such as those with certain heritable cancer syndromes.

### Hormone-Replacement Therapy

Dozens of epidemiological studies have explored possible associations between exogenous estrogens (alone or in combination with progestins) and colorectal neoplasia risk. In a recent meta-analysis of 18 epidemiological studies, the composite RR for ever vs. never users of postmenopausal hormone-replacement therapy was 0.80 (95% CI, 0.74–0.86) for colon cancer and 0.81 (95% CI, 0.72–0.92) for rectal cancer.[214] Current hormone users derived the greatest benefit, a 34% reduction in risk as compared with ever users. Several studies have noted that protective effects against colorectal neoplasia wane after cessation of hormone-replacement therapy, and this further supports a chemopreventive role for estrogen.[214,215]

Few studies have examined the association between estrogen-containing compounds and colorectal adenomas, but available data suggest protective effects against this early phase of carcinogenesis.[216–218] The different mechanisms of chemopreventive activity proposed for estrogens include direct or indirect reductions in secondary bile acid production and inhibition of insulin-like growth factor I, which seems to stimulate epithelial proliferation.[219] The Women's Health Initiative, partially sponsored by the US National Cancer Institute, is the largest trial of hormone-replacement therapy conducted to date.[41] Although the Women's Health Initiative corroborated epidemiological data on the protective effects of estrogen against CRC, in July 2002 the Data Safety and Monitoring Board prematurely halted the placebo-controlled part of the study that compared the use of conjugated equine estrogen plus medroxyprogesterone acetate vs. placebo in 16,608 postmenopausal women with an average follow-up of 5.6 years. This decision was based on an increased overall risk for stroke (stroke hazard ratio [HR], 1.31; 95% CI, 1.02–1.68; ischemic stroke HR, 1.44; 95% CI, 1.09–1.90)[41,218,220] among participants taking conjugated equine estrogen plus medroxyprogesterone acetate.

### Ursodiol

Unconjugated fecal bile acids are mutagenic and cytotoxic and may promote malignant transformation in the colorectum.[221–223] In animal models, deoxycholic acid, a secondary bile salt, has been shown to induce hyperproliferation of colorectal epithelium.[224] In human studies, serum deoxycholate levels correlate with rectal mucosal proliferation and colorectal carcinogenesis.[225]

Ursodiol (also known as ursodeoxycholic acid) is a noncytotoxic, hydrophilic epimer of chenodeoxycholate that has promising chemopreventive efficacy and relatively low toxicity in animal models. Ursodiol-induced prevention of colorectal adenomas and CRCs has been described in several in vivo studies.[226–228] Moreover, ursodiol-induced reductions occur across the spectrum of colorectal neoplasia (e.g., ACF, tumors, and telomerase levels).[229] Ursodiol may effect these changes through enhanced major histocompatibility complex antigen expression, reductions in protein kinase C, stabilization of cellular membranes, and decreased concentrations of mucosal phospholipase $A_2$, prostaglandin $E_2$, and 6-keto prostaglandin $F_{1\alpha}$.[226]

In 1 trial of 19 patients,[230] ursodiol administered for an average of 9 months did not significantly reduce rectal mucosal proliferation. By contrast, in a colonoscopic surveillance study of patients with UC and primary biliary sclerosis, ursodiol use was associated with lower rates of colorectal dysplasia.[231] A prospective trial testing the anti-inflammatory effects of ursodiol in the hepatobiliary tract of 52 individuals with UC and primary sclerosing cholangitis confirmed these findings.[232] In this study, effects against dysplasia and cancer were noted among participants randomized to receive ursodiol vs. placebo (RR, 0.26; 95% CI, 0.06–0.92; $P < 0.03$). It would be premature to generalize these findings to other populations; nevertheless, these mutually confirmatory data strengthen the case for ursodiol as a candidate agent for the chemoprevention of colonic neoplasia in high-risk cohorts, such as those with UC and primary sclerosing cholangitis.[233]

Clinical data regarding the utility of ursodiol for the secondary prevention of colorectal adenoma in postpolypectomy patients will mature over the next few years. Preliminary results from 1 of 2 ongoing trials (Table 6) were recently presented at the 2003 annual meeting of the American Society of Clinical Oncology. Alberts et al. randomized 1285 patients with prior adenomas to ursodiol (8–10 mg $\cdot$ $kg^{-1}$ $\cdot$ $day^{-1}$) or placebo for 3 years and reported statistically insignificant 7% and 14% reductions in patients with recurrent adenomas and advanced adenomas, respectively (Table 5).

### Epidermal Growth Factor Receptor Inhibitors

EGFRs regulate cellular proliferation and differentiation and are highly expressed by many invasive cancers. Hence, EGFR and its ligands are appealing molecular targets, either for direct modulation or indirect control of their downstream effects and signaling pathways.[234] Anti-EGFR monoclonal antibodies and small molecule receptor inhibitors, alone or in combination with other agents, are under evaluation for CRC prevention.

Sulindac combined with a newly developed, irreversible inhibitor of EGFR kinase, EKB-569, was recently shown to be highly protective against intestinal neoplasia in $Apc^{Min/+}$ mice.[185] In that study, untreated $Apc^{Min/+}$ mice developed approximately 20 polyps each, whereas nearly half of the mice treated with the combination regimen developed no polyps. In another preclinical experiment, a different EGFR inhibitor did not reduce the intestinal adenoma burden of Min mice, even at the highest tolerated dose levels.[235] Additional studies will further define the chemopreventive potential of EGFR inhibitors and identify lead compounds within this class of agents.

### Protease Inhibitors

Bowman–Birk inhibitor, a protease inhibitor derived from soybeans, suppresses carcinogen-induced transformation and carcinogenic progression in preclinical studies. Significant reductions in adenomas have been reported in dimethylhydrazine-treated mice administered nontoxic doses of a soybean extract.[236] Bowman–Birk inhibitor inhibits adenoma formation primarily through the inhibition of trypsin.[237] Bowman–Birk inhibitor activity also has been shown in Min mice, where it achieved 42%–50% reductions in tumor multiplicity in both the small intestine and colon, along with a 41% reduction in incident colon cancers.[238] The compound may also reduce neoplastic risk associated with inflammatory bowel disease.[239] Bowman–Birk inhibitor is well tolerated and is now undergoing clinical testing for chemoprevention.[240–242]

### Matrilysin (Matrix Metalloproteinase-7) Inhibitors

Matrix metalloproteinases, including matrilysin, were assumed to operate against advanced stages of carcinogenesis, such as basement membrane invasion and metastasis.[243] As a result, the initial research on this class of agents was directed toward cancer therapy. More recently, Fingelton et al. showed that matrilysin is frequently expressed in adenomas found in *Min* mice and humans, specifically at the luminal surface of dysplastic crypts.[244] In addition, the offspring of matrilysin-deficient mice mated with *Min* mice have 60% reductions in tumor multiplicity and size, showing that matrilysin promotes tumor development, at least in this animal model.[245] Matrilysin is overexpressed in more than 90% of adenomas from FAP patients[246] and in approximately 80% of spontaneous CRCs.[247] Preliminary studies of batimastat (BB-94), a broad-spectrum synthetic matrix metalloproteinase inhibitor with antimatrilysin activity, have shown a 48% reduction in tumor number in *Min* mice. This suggests a possible role for matrix metalloproteinase inhibitors—particularly those targeting matrix metalloproteinase-7—in CRC prevention, either alone[248] or in combination with other chemopreventive compounds.[249]

### 3-Hydroxy-3-Methylglutaryl–Coenzyme A Reductase Inhibitors (or Statins)

3-Hydroxy-3-methylglutaryl–coenzyme A reductase inhibitors are widely used for the primary and secondary prevention of coronary and cerebrovascular diseases. More recently they have been shown to suppress cell growth in several model systems,[250,251] including carcinogen-induced colon tumors in mice.[252,253] Despite a large and growing body of human data on statins, their clinical applications for cancer prevention remain uncertain.

Two meta-analyses of 3-hydroxy-3-methylglutaryl–coenzyme A reductase inhibitors have identified no significant chemopreventive benefits. One meta-analysis of 29,000 subjects treated with 3-hydroxy-3-methylglutaryl–coenzyme A reductase inhibitors for an average of 3.3 years showed no effects on overall cancer incidence (RR, 1.03; 95% CI, 0.90–1.17),[254] and these findings were confirmed by a later meta-analysis.[255] By contrast, secondary analysis of a large Scandinavian randomized, controlled trial of simvastatin showed a 27% reduction in cancer incidence, which was of borderline statistical significance.[256] In addition, a recent analysis of data from the Netherlands associated statins with a significant 20% reduction in overall cancer risk, especially when they were used for $\geq$4 years.[257]

Contradictory data come from the Prospective Study of Pravastatin in the Elderly at Risk, a randomized, controlled study of 5804 men and women 70–82 years of age who were at increased risk for vascular disease.[258] The study participants were randomized to pravastatin 40 mg/day vs. placebo, with a mean follow-up of 3.2 years. Although pravastatin decreased the incidence of the primary composite end point (including coronary heart disease, death, nonfatal myocardial infarction, and stroke), cancer incidence—particularly that of gastroin-

testinal cancers—was higher among participants randomized to pravastatin (overall cancer HR, 1.25; 95% CI, 1.04–1.51; $P = 0.02$; gastrointestinal cancer HR, 1.46; 95% CI, 1.00–2.13; $P = 0.05$). In this elderly cohort over a short observation period, cancer death (6.8% for placebo vs. 8.5% for pravastatin) effectively negated the mortality benefits of pravastatin with regard to coronary heart disease (4.2% for placebo vs. 3.3% for pravastatin). Widespread use for cardiovascular prevention, coupled with mechanistic promise and positive epidemiological data (particularly from studies with long observation periods, which may be essential for showing anticancer effects), compels rigorous clinical testing to evaluate the utility of statins in patients at increased risk for cancer.

## Prospects for Cancer Chemoprevention

The benefits of screening, polypectomy, and surveillance are real, but limited. For example, 4 randomized, controlled trials of fecal occult blood testing documented 15%–30% reductions in CRC mortality.[259] Similarly, although the National Polyp Study showed the preventive efficacy of polypectomy, the precise magnitude of this effect and its relevance to risk reductions in the general population are uncertain. This uncertainty is likely to persist, given that there is little incentive to conduct randomized, controlled trials to quantify it definitively.[260] In addition, recent data heighten concerns about missed lesions, the growth rate of colorectal neoplasia, and the potential of chemotherapeutics to alter the natural history of colorectal neoplasia, as well as the limitations of current endoscopic detection methods. One cancer cooperative group trial reported a rate of metachronous cancers that is 6.8 times higher than that in the National Polyp Study[261]; also, the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial recently reported nearly a 1% incidence of advanced adenomas or cancers in the distal colon among patients who had a flexible sigmoidoscopy 3 years earlier.[262] Finally, in very-high-risk groups, complete colonoscopic surveillance may be technically challenging or impossible. Therefore, for the foreseeable future, complete surgical colectomy remains the safest approach to high-risk disease management.[263] Conceivably, cancer chemoprevention will complement or ultimately replace conventional screening and detection strategies, thereby improving clinical and public health outcomes within and across these and other cohorts at risk for CRC.

Many opportunities to identify or prioritize promising compounds exist but have yet to be adequately explored. Endoscopic surveillance nested within postlicensure studies or during clinical testing of new agents intended for other indications might provide concomitant, albeit preliminary, information on agent efficacy for cancer chemoprevention.[34] For example, PPAR-γ ligands such as troglitazone, pioglitazone, and rosiglitazone are currently used for glycemic control in millions of non–insulin-dependent diabetics. PPAR-γ ligands regulate adipocyte growth arrest, cellular proliferation, and differentiation, and their chemopreventive potential was recently shown in ACF rat models.[264,265] Diabetics are at increased risk for CRC, and observational studies of neoplastic outcomes in this population might justify prospective testing of PPAR-γ ligands for cancer chemoprevention. Although CRC incidence may be rare in these trials, adenoma incidence may be a reasonable secondary end point. Such assessments are technically and ethically feasible and may suggest trial designs and appropriate cohorts for future research. Likewise, a registry that collects CRC screening and surveillance data on individuals taking PPAR-γ ligands would provide valuable insights into the potential activity of these agents against colorectal neoplasia. More broadly, such approaches could be used to assess the preliminary efficacy of compounds commonly used and tested for other indications, such as statins and nutritional supplements. Treatment trials, including those testing vaccines and monoclonal antibodies, provide additional—and largely unexplored—opportunities to identify promising agents for CRC chemoprevention.[266]

Clinical trials are the best means to identify the most promising chemopreventive agents, schedules, and doses, as well as the cohorts most likely to benefit from them. Although 30% of the US population over age 50 may harbor adenomas and although adenomas may recur within 1–3 years in 30% of patients, phase III trials typically take more than 5 years to complete, largely owing to accrual difficulties. Slow or inadequate participant recruitment is a perennial problem that stymies the timely execution of most cancer-prevention trials. Research networks dedicated to CRC prevention might accelerate accrual onto trials by facilitating access to at-risk patients and tissue acquisition/analysis within and across institutions, disciplines, and projects. Gastroenterologists are uniquely poised to advance this effort but need a sustainable infrastructure to support it. Federal agencies are exploring how to design and fund a gastroenterology research network that might facilitate cancer prevention research (http://prg.nci.nih.gov/stomach/finalreport.html).

Sixty percent of the eligible US population has never undergone CRC screening, even though it has been proven effective.[267] Although the profile of agents acceptable for populations at high vs. average risk for CRC is likely to differ, chemopreventive agents may help to address the vast gap between medical logic and individual behavior. Among cohorts at average to moderate risk for CRC, successful chemopreventive agents are likely to be essentially nontoxic, effective against many common cancers, or capable of modulating many chronic diseases of aging (e.g., atherogenesis, carcinogenesis, osteoporosis, and inflammation). This is no longer mere conjecture—the efficacy of aspirin and calcium across a broad range of diseases common to Western populations suggests that conditions formerly regarded as biologically unique may share critical molecular determinants.

## Conclusions

New technologies have made CRC risk assessments more sensitive and reliable and have brought an increasing number of colorectal neoplasias to clinical presentation.[268] Within the last century, cancer and cardiovascular disease surpassed infectious disease as the major causes of morbidity and mortality in Western societies. Cancer and cardiovascular disease rates are bound to increase, because by 2030 the US population over the age of 65 is expected to double and the population over the age of 85 to quadruple.[269] Molecular prevention will become increasingly valued as we confront the wide spectrum of health-care challenges posed by this demographic shift.

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**Received July 9, 2003. Accepted November 13, 2003.**

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**This article was prepared in our capacity as employees of the U.S. Federal Government.**