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# New Agents for Cancer Chemoprevention

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**Abstract:** Clinical chemoprevention trials of more than 30 agents and agent combinations are now in progress or being planned. The most advanced agents are well known and are in large Phase III chemoprevention intervention trials or epidemiological studies. These drugs include several retinoids [*e.g.*, retinol, retinyl palmitate, all-*trans*-retinoic acid, and 13-*cis*-retinoic acid], calcium, βcarotene, vitamin E, tamoxifen, and finasteride. Other newer agents are currently being evaluated in or being considered for Phase II and early Phase III chemoprevention trials. Prominent in this group are all-*trans*-*N*-(4-hydroxy phenyl)retinamide (4-HPR) (alone and in combination with tamoxifen), 2-difluoromethylornithine (DFMO), nonsteroidal antiinflammatory drugs (aspirin, piroxicam, sulindac), oltipraz, and dehydroepiandrostenedione (DHEA).

A third group is new agents showing chemopreventive activity in animal models, epidemiological studies, or in pilot clinical intervention studies. They are now in preclinical toxicology testing or Phase I safety and pharmacokinetics trials preparatory to chemoprevention efficacy trials. These agents include *S*-allyl-*l*-cysteine, curcumin, DHEA analog 8354 (fluasterone), genistein, ibuprofen, indole-3-carbinol, perillyl alcohol, phenethyl isothiocyanate, 9-*cis*-retinoic acid, sulindac sulfone, tea extracts, ursodiol, vitamin D analogs, and *p*-xylyl selenocyanate. A new generation of agents and agent combinations will soon enter clinical chemoprevention studies based primarily on promising chemopreventive activity in animal models and in mechanistic studies. Among these agents are more efficacious analogs of known chemopreventive drugs including novel carotenoids (*e.g.*, α-carotene and lutein). Also included are safer analogs which retain the chemopreventive efficacy of the parent drug such as vitamin $D_3$ analogs. Other agents of high interest are aromatase inhibitors (*e.g.*, (+)-vorozole), and protease inhibitors (*e.g.*, Bowman-Birk soybean trypsin inhibitor). Combinations are also being considered, such as vitamin E with *l*-selenomethionine. Analysis of signal transduction pathways is beginning to yield classes of potentially active and selective chemopreventive drugs. Examples are *ras* isoprenylation and epidermal growth factor receptor inhibitors. 1997 Wiley-Liss, Inc.*

**Key words:** cancer chemopreventive agents, drug development, retinoids, DFMO, NSAIDs, oltipraz, Phase I clinical trials, Phase II clinical trials

Chemoprevention drug development has the goal of identifying safe and effective chemopreventive agents for clinical use. The NCI's chemoprevention drug development program, which has been described previously [1–7], is an applied drug development science effort. This program begins with identifying candidate agents for development and characterizing these candidates for efficacy using mechanistic assays and *in vitro* and animal chemopreventive efficacy screens. Since the program's inception in 1987, more than 400 agents have entered the program. Of these, more than 250 have been tested or are currently on test in the animal screens. Promising agents are then further evaluated in animal models to design regimens for clinical testing and use. Agents judged to have potential as human chemopreventives are subjected to preclinical toxicity and pharmacokinetic studies, and then Phase I clinical safety and pharmacokinetic trials. The most successful agents then progress to clinical chemoprevention trials; more than 30 agents are now being studied in Phase II and III trials. The purpose of this paper is to discuss the strategies, perspectives, and progress of some of the most promising of these new chemopreventive agents—both those already in chemoprevention trials and those emerging from preclinical and clinical safety and pharmacokinetics testing, as well as those showing promising activity in

preclinical efficacy and mechanistic studies.

## PHASE II CHEMOPREVENTION TRIALS—SURROGATE ENDPOINT BIOMARKERS

Besides well-characterized agents, suitable cohorts and reliable intermediate biomarkers for measuring efficacy are required for successful chemoprevention trials. A significant aspect of our chemoprevention program is the development of Phase II clinical protocols using intermediate biomarkers to serve as surrogate endpoints for cancer incidence. Particularly important are cellular morphologic and densitometric changes that define intraepithelial neoplasia (IEN). IEN changes are on the causal pathway to cancer [8,9]. They may serve as target lesions in Phase II chemoprevention trials and as standards against which to evaluate other earlier cellular and molecular biomarkers—proliferative lesions and signals, differentiation signals, and genetic changes such as DNA content and oncogene and tumor suppressor expression.

One criterion for selecting cohorts in chemoprevention trials is expectation of a high incidence of the cancer or intermediate biomarker(s) under study within a reasonable time period. For Phase II studies, the study duration should be ≤2 years, and for Phase III trials, ≤10 years. Some patients previously treated for cancer are good candidates for chemoprevention trials because of high rates of recurrence or second primary cancers. We have defined strategies for the clinical evaluation of chemopreventive agents in ten targets—prostate, breast, colon, lung, bladder, cervix, esophagus, oral cavity, skin, and liver. In these targets, cohorts have been identified for short-term Phase II trials that investigate the effects of chemopreventive agents on IEN and earlier biomarkers. Patients with adenomas serve as a cohort for trials in colon. One cohort for Phase II trials in prostate is early stage cancer patients scheduled for prostatectomy; another is patients with prostatic intraepithelial neoplasia (PIN), but without prostatic carcinoma. Patients treated for lung cancer are at high risk for bronchial dysplasia and second cancers; such patients are a cohort for Phase II trials in lung cancer, as are chronic smokers with bronchial dysplasia. Patients with Barrett's esophagus form a cohort for chemoprevention studies of esophageal cancer. Presurgical breast cancer patients and patients with ductal carcinoma *in situ* (DCIS) are cohorts for studies in breast. Subjects at high risk for breast cancer who also show multiple biomarker abnormalities may also be a cohort for Phase II studies in breast [10]. Patients with superficial bladder cancers (Ta/T1 with or without carcinoma *in situ*) are cohorts for studies of chemoprevention in bladder, and patients with dysplastic oral leukoplakia are evaluated for chemoprevention of oral cancers. Cervical intraepithelial neoplasia (CIN) is a prototype IEN, and patients with CIN are a cohort for studies of cervical cancer.

## AGENT COMBINATIONS

Cancer incidences may not be reduced to the full extent possible by single agents. More commonly, very promising agents show significant toxicity at efficacious doses. The simultaneous or sequential administration of multiple inhibitors can increase efficacy and reduce toxicity. Such an approach uses differences in the mechanisms of cancer inhibition among the agents to increase inhibitory activity. Further, the increased efficacy achieves desirable levels of cancer inhibition at lower and presumably less toxic doses of the individual agents.

Positive effects have been demonstrated in animal models using combinations of chemopreventive agents. Several have shown synergism, *i.e.*, the inhibitory potency of the combination was greater than the sum of the potencies of the single agents. Synergistic chemopreventive activity has been reported for 2-difluoromethylornithine (DFMO) and piroxicam in rat colon [11,12] and for all-*trans*-*N*-(4-hydroxyphenyl)retinamide (4-HPR) and tamoxifen in rat mammary gland [13,14]. In other studies, synergistic activity has been observed in hamster lung for β-carotene with 4-HPR and with vitamin A and for 4-HPR with oltipraz [15]. Likewise, combinations of DFMO with 4-HPR and with oltipraz, and 4-HPR with oltipraz have synergistic activity in the bladder [16]. A Phase II study with 4-HPR and tamoxifen is currently in progress in breast. As will be described below, the combination of DFMO with piroxicam is in Phase I trials, preparatory to a Phase II study in prevention of colon adenomas. Also, several other combinations are currently undergoing toxicology testing in our chemopreventive drug development program based on promising results in animal cancer models or mechanism of action. An interesting example of using mechanistic considerations is the combination of *N*-acetyl-*l*-cysteine (NAC) and oltipraz. Both agents enhance glutathione (GSH) carcinogen detoxification pathways. NAC is a cysteine prodrug

[17], and thereby provides substrate for the biosynthesis of GSH. Oltipraz stimulates GSH-*S*-transferases (GST) and other Phase II enzymes which catalyze the formation of GSH-carcinogen detoxification products [18].

## CHEMOPREVENTIVE AGENTS IN PHASE II/III CLINICAL TRIALS

Of particular interest are the retinoids which have demonstrated chemopreventive activity in clinical settings. For example, 13-*cis*-retinoic acid inhibited the formation of second primary tumors in patients with previously resected head and neck cancers [19] and oral leukoplakia [*e.g.*, 20,21] and prevented squamous cell carcinoma of cervix and skin when administered with IFN-α [22]. In a limited study, all-*trans*-retinoic acid caused regression of cervical intraepithelial neoplasia [23]. Retinoids are active in the proliferation and progression stages of carcinogenesis [24]. They inhibit several activities involved in tumor promotion, including induction of ornithine decarboxylase (ODC), and participate in signal transduction via cellular receptors. They induce terminal differentiation in selected cells, and this activity may be mediated by interaction with cellular receptors. Particularly, retinoids have been shown to reduce circulating IGF-I [25] and induce specific isoforms of TGFβ [26,27]. They also induce apoptosis [28,29] stimulate intercellular communication [29] and are immunostimulants [29].

*4-HPR* is a newer retinoid. The well-known Phase III trial of 4-HPR in previously treated breast cancer patients to evaluate chemoprevention of second primary breast cancer began in March 1987 (Dr. Umberto Veronesi, Istituto Nazionale Tumori). Patients are given 200 mg 4-HPR qd for five years with two years follow-up and compared with untreated controls. A recent interim analysis showed decreased incidences of second primary tumors in premenopausal women treated with 4-HPR; also, fewer 4-HPR treated patients than controls have developed ovarian cancers [30,31]. 4-HPR is also currently being tested in Phase II trials in breast (alone and in combination with tamoxifen), cervix, lung, prostate, bladder, skin (actinic keratosis patients), and oral cavity.

Since chemopreventive agents are intended for chronic use in healthy or relatively healthy subjects, toxicity, even if mild and reversible, is problematic. Often, drug development for such agents involves strategies for reducing these toxicities. 4-HPR, like other retinoids, causes ophthalmologic disturbances (primarily reduction in night vision) due to depletion of retinal vitamin A. In the clinical trials with 4-HPR, the strategy for alleviating toxicity is two- or three-day drug holidays monthly or biweekly to allow recovery of vitamin A levels.

*DFMO* alkylates and irreversibly blocks ODC, preventing conversion of ornithine to putrescine. This is the first and rate-limiting step in polyamine synthesis, which is closely linked to cell proliferation [32–34]. ODC is believed to be important in tumor promotion [35,36], and its inhibition thus may be a mechanism for inhibiting carcinogenesis. DFMO has chemopreventive activity in mouse skin, mouse colon, rat colon, rat liver and stomach, rat and mouse urinary bladder, and rat mammary gland [37]. As noted above, it also has synergistic efficacy with piroxicam in rat colon; oltipraz in mouse bladder; and 4-HPR in mouse bladder and hamster lung. Recently, it was found to have synergistic efficacy with 4-HPR in inhibition of lymphoma in PIM-1 transgenic mice. Because only 50% of an oral dose is absorbed, and 80% of that absorbed is excreted unchanged in the urine [38], colon and bladder cancers are considered primary targets for chemoprevention by DFMO.

The most frequently observed clinical side effect of DFMO administration is reversible ototoxicity. Phase I clinical trials showed drug effect with no toxicity, particularly otoxicity, in patients treated with 0.5 g/m$^2$/day (13 mg/kg-bw/day) for 10–12 months [37, 39], suggesting that this dose level is appropriate as a starting point for further clinical studies. Since this baseline dose may prove too high for continuous exposure, a high priority in Phase II trials is dose-titration to determine the lowest efficacious dose. DFMO is being evaluated in Phase II studies in cervix, bladder, breast, prostate, oral leukoplakia, and Barrett's esophagus.

Three *nonsteroidal antiinflammatory drugs (NSAIDs)—sulindac, piroxicam, and aspirin*—are in Phase II chemoprevention trials (a fourth NSAID, ibuprofen, has not yet been evaluated in Phase II efficacy studies). A prominent biological activity of the NSAIDs is inhibition of the synthesis of prostaglandins and other eicosanoids, particularly by inhibition of fatty acid cyclooxygenase (COX) [*e.g.*, 40–42]. Epidemiological and experimental data strongly suggest that carcinogenesis in epithelial tissues may be modulated by inhibiting some aspects of

the prostaglandin biosynthetic cascade [*e.g.*, 40–43]. The mechanism(s) may involve reductions not only in growth-promoting tissue prostaglandin levels but also in suppressed immune surveillance [44,45], aspects of signal transduction such as *ras* oncogene activation [46] and oxidation (activation) of proximate carcinogens [43,48].

In animal studies, NSAIDs have chemopreventive activity in numerous tissues [4,49–52]. They reduce formation of both colon polyps and carcinomas in laboratory animals given carcinogens. They also inhibit the induction of tumors in rat urinary bladder, hamster buccal pouch, rat mammary gland, mouse skin and duodenum, and hamster esophagus, pancreas, and uterine cervix.

In animal efficacy screens carried out in our drug development program [53], the NSAIDs were active in the rat colon (aspirin, ibuprofen, piroxicam, sulindac), rat mammary gland (ibuprofen), mouse bladder (ibuprofen, piroxicam, sulindac) and mouse skin (piroxicam). The highest chemopreventive potency of NSAIDs in the animal models appears to be in colon and bladder, and so these are the primary targets in clinical trials. In preliminary clinical studies, sulindac has also shown dramatic effects in causing the total or almost total regression of colorectal adenomatous polyps in patients with familial adenomatous polyposis (FAP) and Gardner's syndrome [54, 55]. Epidemiological studies also suggest that NSAIDs have promise in the clinic as colon cancer chemopreventives. For example, regular aspirin use (≥16x/month) has been reported to reduce the relative risk of death from colon cancer by 40% [56]. Recent analysis of data on women in the Nurses' Health Study showed reduced risk of colorectal cancer associated with ≥10 years of regular aspirin use (4–6 tablets/week) [57]. Phase II trials of sulindac and piroxicam in preventing colon polyps are in progress [52]. Several trials with aspirin in colon have been completed or are ongoing [49]. Particularly interesting in light of the results of the Nurses' Health Study is a Phase III trial evaluating the chemopreventive effects of 100 mg aspirin qod for four years on the incidence of epithelial cancers (breast, colon, and lung) in female health professionals ≥45 years old (Dr. Julie Buring, Brigham and Woman's Hospital, Boston) [see 49]. Vitamin E at 600 IU is being evaluated in the same study.

For clinical use as chemopreventives, the goal is to identify doses/regimens for NSAIDs which are efficacious and nontoxic with respect to the gastric bleeding, ulcers, and nephropathy which are caused by local inhibition of prostaglandin $E_2$ ($PGE_2$) and which limit NSAID usage in other applications. Several possible strategies are being explored. One possibility is combination with other agents to allow chemopreventive intervention at lower and presumably less toxic doses. An example noted above is the combination of the NSAID piroxicam with DFMO. The objective of the Phase I trial of this agent combination is to define a safe efficacious dosing regimen for a Phase II trial in the prevention of colon polyps.

Another strategy is the use of agents which retain NSAID ability to inhibit the carcinogenesis-associated activities of prostaglandin synthesis without depressing its protective effects. The discovery of an inducible form of COX (COX-2), which is predominant at inflammation sites and in macrophages and synoviocytes, suggests that such an approach is feasible [58]. In contrast to COX-2, constitutive COX-1 predominates in the stomach, gastrointestinal tract, platelets, and kidney. The NSAIDs currently under development inhibit both forms of the enzyme, but other compounds inhibit COX-2 selectively (*e.g.*, NS-398). Although no positive animal test results are yet available, such agents may prove to be desirable alternatives to the NSAIDs currently being developed.

A third strategy is administering the chemopreventive NSAID with a drug which protects the gastric mucosa. One such drug is the prostaglandin derivative misoprostol. A fourth strategy is use of metabolites or other derivatives of NSAIDs which retain efficacy, but reduce the associated toxicity. An example is the sulindac metabolite, sulindac sulfone. This agent is currently in a Phase I/IIa safety and preliminary efficacy study in patients with FAP and is described below.

*Oltipraz* is a synthetic dithiolthione related to naturally occurring 1,2-dithiolthiones found in cruciferous vegetables. It is a schistosomicidal drug that has demonstrated chemopreventive efficacy in many animal model systems. Oltipraz inhibited the induction of mouse forestomach and lung tumors, aflatoxin $B_1$ ($AFB_1$)-induced rat liver cancer, azaserine- induced rat pancreatic cancer, and spontaneous rat hematopoietic tumors [59]. Oltipraz has been highly effective in animal screens carried out under our drug development program [53]—positive results have been seen in hamster lung and trachea [15], mouse

and rat colon [60,61], rat mammary, mouse bladder [16], mouse skin, and rat thymic lymphomas induced by PhIP [62].

Although the mechanism of this activity is not fully understood, the anticarcinogenic potential of oltipraz was first suggested by its chemoprotective, radioprotective, and antimutagenic properties [63, 64]. The agent also inhibited $AFB_1$-induced hepatotoxicity and DNA adduct formation in rat liver [64]. Orally administered oltipraz increases liver GSH levels and induces enzymes involved in electrophile detoxification—namely, GST, epoxide hydrolase, and NAD(P)H:quinone oxidoreductase [63–65]. GSH is present in high concentrations in most cells, where it functions to inactivate electrophilic carcinogens and scavenge oxygen free radicals. It also reacts with hydrogen peroxide catalyzed by GSH peroxidase (GSH-Px) and prevents the formation of other more reactive oxygen compounds [17]. The chemopreventive and chemoprotective efficacy of oltipraz in liver has been attributed to these activities [63, 66,67]. There also is some evidence that oltipraz may have antiproliferative effects that may or may not be directly related to modulation of GSH and the phase II metabolic enzymes. For example, in our efficacy studies in rat colon [53,61] and mammary gland [53], the agent was effective even when it was administered only after treatment with the carcinogen had been completed.

A Phase I MTD of approximately 125 mg (*ca.* 0.008 mmol/kg-bw) qd has been identified [59,68]. Recent evidence indicates that intermittent dosing is feasible [69]. A pharmacokinetic profile is now being evaluated for this highly lipophilic drug [68]. Despite these encouraging results, much work remains to be done to determine the appropriate chronic clinical dosage regimen for chemoprevention studies. The agent has shown significant toxicity in certain clinical settings. For example, in a six-month Phase I clinical trial at 125 and 250 mg/day, side effects included photosensitivity, heat intolerance, gastrointestinal discomfort, neurological abnormalities, and an altered taste; the lower dose was considered to be in excess of the MTD [68].

The agent is being evaluated in Phase II trials in liver and lung. The intervention phase of the trial in liver has been completed. This study was conducted in subjects in the Qidong region of China (Dr. Thomas W. Kensler, Johns Hopkins University), who are exposed to the liver carcinogen $AFB_1$ and in whom the liver cancer predisposing hepatitis B virus infection is endemic. The primary endpoints of the trial are carcinogen effect biomarkers—inhibition of $AFB_1$-DNA and -protein adducts. Dosing was 125 mg qd or 250 mg 2x/week. As in other human studies, gastrointestinal distress and side effects related to photosensitivity are prevalent (≥10% of trial population) [70]. Also, an adjunct to this chemoprevention study will be the evaluation of another exposure biomarker, urinary mutagens, in the subject population (Dr. Silvio De Flora, University of Genoa). Another important aspect of oltipraz is its potential effect in patients infected with human immunodeficiency virus type 1 (HIV-1). GSH levels are depleted in such patients and it has been found that oltipraz can elevate GSH levels in human T-cell and monocytoid cell lines and inhibit HIV-1 replication [71]. It both inhibits reverse transcriptase and HIV-1 infection in a chronic infection model. Prochaska [71] considers that it has potential for inhibiting HIV-1 associated neoplasms along with its antiretroviral activity.

*Dehydroepiandrostenedione (DHEA).* Schwartz and his colleagues, as well as other investigators, have demonstrated the chemopreventive activity of the androgen DHEA in numerous animal models [72]. In our studies, DHEA was efficacious in mammary gland, prostate and colon models of carcinogenesis [53]. In MNU-induced rat mammary gland, DHEA at 1 or 2 g/kg diet (*ca.* 0.2 or 0.4 mmol/kg-bw/day), given one week prior to MNU and continued for the remainder of the experiment, reduced both the incidence and multiplicity of histologically confirmed carcinomas [73]. A more recent study in MNU-induced rat mammary glands indicated efficacy for DHEA at lower doses of 120 or 600 mg/kg diet (*ca.* 0.02 mmol or 0.1 mmol/kg-bw/day, respectively) [74]. The 0.1 mmol/kg-bw/day dose inhibited tumor incidence, multiplicity and number, while the 0.02 mmol/kg-bw/day dose inhibited only tumor multiplicity and number. One (1) and 2 g DHEA/kg diet (*ca.* 0.17 and 0.35 mmol/kg-bw/day) were found to significantly decrease the incidence of macroscopic cancers in the MNU-induced, hormone-promoted rat prostate. In the MAM-acetate-induced mouse colon cancer model, DHEA inhibited colon cancer at the lowest dose tested—0.15% DHEA in diet (*ca.* 0.6 mmol/ kg-bw/day).

DHEA is a potent inhibitor of glucose-6-phosphate dehydrogenase (G6PDH). The primary function of this enzyme is catalyzing the formation of

extramitochondrial NAD(P)H and ribose 5-phosphate. Schwartz has hypothesized two ways in which inhibition of G6PDH may mediate the chemopreventive activity of DHEA [75]. First, DHEA inhibits the activity of carcinogens such as B(*a*)P, $AFB_1$, and DMBA which require metabolic activation via mixed function oxidases (MFO) [75–78]. MFO require NAD(P)H as a cofactor. Thus, since inhibition of G6PDH reduces the formation of NAD(P)H, it consequently reduces the activity of MFO and the activation of certain carcinogens. Secondly, DHEA also inhibits tumor promotion and proliferative activity [79,80]. Cell proliferation requires NAD(P)H-dependent DNA synthesis, and DNA synthesis in mouse epidermis and mammary tissue also is inhibited by DHEA [81]. Accordingly, reduction of the NAD(P)H pool by inhibition of G6PDH could inhibit carcinogen-induced cell proliferation. Recent evidence indicates that DHEA may modulate signal transduction pathways. Particularly, DHEA inhibits the posttranslational farnesylation and activation of *ras* oncogene [82,83].

Unfortunately, the chemopreventive potential of DHEA is compromised by some undesirable pharmacological effects—potent hormonal [84], liver-enlarging [85], and peroxisome-proliferating activities [85,86]. Also, chronic administration of DHEA in the diet has been shown to reduce body weight without reducing food intake, although this problem can be negated with lower dosing [75]. For example, in our rat mammary gland studies cited above, the 0.4 mmol/kg-bw/day dose produced significant weight loss relative to carcinogen controls (10%). If this dose was given one week after MNU and continued for the duration of the experiment, significant reduction of carcinoma incidence and multiplicity was observed without weight loss. When the high dose was given only one week prior to and one week following MNU, DHEA had no effect on tumor incidence or multiplicity, nor on body weight. In the study with effective doses of 0.1 and 0.2 mmol/kg-bw/day, neither dose caused changes in body weight relative to carcinogen-treated controls. Also, in the prostate cancer model, no effect on body weight gain was seen. Thus, it may be possible to find effective doses that do not alter body weight.

Short-term Phase II chemoprevention trials will be initiated in presurgical breast DCIS and prostate cancer patients. The dose selected for these trials, 300 mg qd (*ca.* 0.016 mmol/kg-bw/day) has been administered to postmenopausal women in previous Phase I safety studies [87]. The consensus from these and other studies is that orally administered DHEA is well tolerated by men and women at doses up to *ca.* 0.08 mmol/kg-bw/day for one month. At lower doses (*ca.* 0.002 mmol/kg-bw/day), DHEA is well tolerated for six months [88–90].

Another strategy to eliminate DHEA side effects while preserving chemopreventive activity has been pursued by Schwartz who designed several analogs [75,85,91]. One of these analogs, DHEA analog 8354 is particularly promising, and is also being developed in the NCI chemopreventive drug program (see description below).

## CHEMOPREVENTIVE AGENTS IN PHASE I CLINICAL TRIALS OR PRECLINICAL TOXICOLOGICAL STUDIES

S-*Allyl*-l-*cysteine* is a water soluble organosulfur compound found in garlic. For many years, there has been a high level of interest in the potential chemopreventive effects of garlic, onion and their components [92]. Epidemiological studies have shown inverse correlations between gastric cancer incidence and consumption of vegetables in the *Allium* genus [93,94]. Garlic oil has shown chemopreventive activity in mouse skin [95] and cervix [96], and several of its volatile, lipophilic components (particularly, diallyl sulfide) have shown chemopreventive activity in mouse colon and stomach [97–99], mouse skin [100], rat esophagus [101], rat glandular stomach [102], and hamster trachea [53].

The volatility and pungency of the lipophilic garlic compounds make them difficult to test and unpalatable. These disadvantages have led to interest in the water soluble, less aromatic components such as *S*-allyl-*l*-cysteine. On oral administration the compound inhibited DMH-induced colon tumors in female C57BL mice [103].

The mechanism of action of the garlic sulfur compounds is not well-understood but appears to be related to electrophile detoxification. Like oltipraz and the structurally closely-related NAC, *S*-allyl-*l*-cysteine [103] and other garlic sulfur compounds [98] enhance GST activity. Also, diallyl sulfide and, probably, other garlic sulfides inhibit cytochrome P450IIE1 which is involved in metabolic activation of carcinogens such as DMH and MBN [92,104]. Preclinical acute and subchronic (90-day) toxicity evaluations of *S*-allyl-*l*-cysteine in rats and dogs have

been completed. In rats, the NOEL in the 90-day studies was 125 mg/kg-bw/day for females (no NOEL was determined for males). In both male and female dogs, 30 mg/kg-bw/day was determined to be the NOEL. In both species, kidney toxicity was evident in these studies. However, as for NAC, the clinical toxicity of *S*-allyl-*l*-cysteine is anticipated to be very low. A Phase I clinical trial of *S*-allyl-*l*-cysteine is planned.

*Curcumin* is the major yellow pigment in turmeric and curry and is obtained from the rhizome of the plant *Curcuma longa*. In our studies, curcumin had chemopreventive activity in mouse colon and MNU-induced rat mammary models [53]. In other published studies, the agent had tumor inhibitory activity in mouse skin [105–107].

Curcumin has multiple potential chemopreventive mechanisms. It is a potent antiinflammatory agent [108–112]. It inhibits arachidonic acid metabolism by blocking both the lipoxygenase and COX pathways [108,113], and, possibly, phospholipase $A_2$ [108]. Curcumin exhibits strong antioxidant activity [114,115], being an effective superoxide scavenger [116]. It inhibits TPA-induced DNA synthesis, demonstrating an inhibitory effect on proliferation [106]. It also modifies cytochrome P450 [117] and enhances GST activity [118,119], and it may modify the metabolic activation and DNA binding of PAH carcinogens by this mechanism [108,120,121]. It inhibits mutagenesis [122,123] and clastogenesis [117,124,125]; decreases expression of c-*jun* [126, 127], c-*fos* [127] and c-*myc* [127], possibly through inhibition of protein kinases [128]; inhibits ODC activity [*e.g.*, 129] and EGFR function [130,131]; and enhances DNA repair [132]. It may also have antihormonal action, since extracts of turmeric were reported to decrease testosterone production in male rats and implantation in female rats [133], as well as antiviral activity [134]. As noted above, curcumin is not expected to exhibit much toxicity in humans. Toxic effects of chronic human exposure have not been characterized apart from respiratory symptoms and allergic dermatitis in spice factory workers [135]. Consistent with inhibition of prostaglandin synthesis, ulcerogenic effects have been reported in rats [136] and mice. In mice, these effects were seen only at doses double the median effective antiinflammatory dose (0.15 mmol/kg-bw/day for six days). In our acute toxicity study in rats, curcumin was not toxic—*i.e.*, $LD_{50}$ >3.5 g/kg-bw, the highest dose that reasonably could be administered orally. Subchronic studies (up to 90 days) in rats, dogs and monkeys generally showed limited adverse effects. For example, in our 90-day studies of commercial grade curcumin, minor changes in body weights in rats and hematological values in rats and dogs were not considered biologically significant. A Phase I safety and pharmacokinetics study of curcumin will start soon. It is likely that both food grade (85% pure) and purified curcumin will be evaluated.

As noted above, *DHEA analog 8354 (fluasterone, 16α-fluoro-DHEA)* does not have the androgenic or liver toxicity of DHEA [85]. It was a more potent inhibitor of tumor initiation and promotion in the DMBA/TPA mouse skin model than DHEA [80], and, in our animal studies, it was effective in the rat mammary gland against MNU-induced cancers [137, 138] and in rat colon against AOM-induced tumors [12,137].

Subchronic toxicity studies in rats and dogs have established a NOEL of 250 mg/kg-bw for DHEA analog 8354 in both species; no target organs with histopathology were identified in either study. Effects seen at the high doses tested included dose-related weight loss (>10% at 1 g/kg-bw/day) and hypocholesterolemia (at 500 mg/kg-bw/day and 1 g/kg-bw/ day) in the male rats. The relevance of these effects to the potential of the analog for clinical use has not yet been evaluated. Particularly, the minimal effective doses of the compound have not been determined. Pharmacokinetic evaluations are currently underway and chronic toxicity studies are planned. DHEA analog 8354 is also being reformulated to augment bioavailability.

*Genistein.* The isoflavone genistein occurs naturally in soybeans [139] and some forage plants [*e.g.*, 140,141] as the glucoside, and has been identified in soy food products and beer [142,143]. Epidemiological studies have shown an association between decreased hormone-dependent cancer (*e.g.*, in breast and prostate) incidence/mortality and a traditional soy-rich Asian diet [144]. Individuals consuming this diet have 7–110-fold higher plasma [145] and 30-fold higher urinary [146,147] genistein concentrations than individuals consuming a typical Western diet. A tentative causal relationship is suggested by limited studies showing the inhibitory effects of dietary soy products in chemical- [143,148,149] and radiation-[150,151] induced rat mammary and estrogen-induced mouse prostate models of carcinogenesis [152].

Preclinical efficacy studies to date have produced inconsistent results, perhaps due to the pleiotropic activities of genistein. In the AOM-induced rat, genistein at 75 and 150 mg/kg diet (*ca.* 13.8 and 27.8 μmol/kg-bw/day) inhibited the number of aberrant crypt foci (ACF)/colon, histological intermediate biomarkers in the AOM-induced rat model of colon carcinogenesis [153,154]. The number of aberrant crypts/focus was not reduced. In contrast, in the same model, 250 ppm genistein in diet had no effect on colon or small intestine tumor incidence, and significantly increased colon tumor multiplicity compared with controls [155].

*In some* situations, the potential chemopreventive efficacy of genistein appears to be related to its phytoestrogenic effects. Genistein competes with estradiol for estrogen receptors [156,157] and the complex translocates to the nucleus [158], stimulating estrogen-related cellular events [141,159–161]. For example, genistein decreases the formation of DMBA-induced rat mammary tumors, possibly by accelerating cellular differentiation [162, ,163], and a reduction in DMBA-DNA adducts in mammary gland tissue has also been demonstrated [164]. Based on preliminary data, genistein was not effective in the MNU-induced rat mammary model [165], but the tested dose (0.8 mg/day ig) was much lower than those found to be effective in the immature rats given DMBA or the estimated dose from dietary soybean protein in the MNU model [*i.e.*, 149]. However, estrogen is generally considered to enhance hormone-related tumorigenesis, especially in the breast and endometrium [166]. Genistein may have either estrogen-dependent or -independent effects, depending on its concentration or the assay used. In human breast cancer MCF-7 cells, genistein stimulated both cell growth and estrogen-dependent pS2 expression at low concentrations; however, it inhibited cell growth at higher ($>10^{-5}$ M) concentrations even though pS2 was still expressed [167].

The major competing estrogen-independent mechanism is inhibition of tyrosine-specific protein kinase activity [168,169]; this may in turn inhibit this may in turn inhibit cell proliferation [170,171] and growth factor-stimulated responses (EGFR, IGF-I, PDGF) [168,172, 173], oncogene product activity ($pp60^{v\text{-}src}$, $pp110^{gag\text{-}fes}$, $p210^{c\text{-}abl}$) [174,175] or expression (*ras*, c-*fos*, c-*jun*) [168,176], bFGF-induced angiogenesis [*e.g.*, 177], prostaglandin synthesis [*e.g.*, 178,179], DNA synthesis [*e.g.*, 180], ODC activity [*e.g.*, 181], and immune response [182], as well as induce differentiation [*e.g.*, 162,183]. Other potentially chemopreventive activities which appear to be estrogen-independent include inhibition of cytochrome P450 metabolism [184,185], scavenging reactive oxygen species [*e.g.*, 186–188], and topoisomerase activity [189], as well as induction of cell cycle arrest [190,191] and apoptosis [192–194].

Because of its multiple potentially chemopreventive activities and evidence of its apparent bioavailability on oral administration [145–147], we are now evaluating the preclinical toxicity of genistein as a prerequisite for Phase I clinical studies. We have undertaken 90-day preclinical toxicity tests with two purified soy isoflavone products containing 90% genistein and 43% genistein in rats (*ca.* 0.03– 0.9 mmol genistein/kg-bw/day) and dogs (*ca.* 0.02–0.3 mmol genistein/kg-bw/day). In the recently completed dog study, no significant toxicity was observed.

*Ibuprofen.* The suggested therapeutic doses of this NSAID are 1,200–3,200 mg/day (*ca.* 17–45 mg/kg-bw/day), and its gastrointestinal toxicity appears to be less severe than that of aspirin and sulindac. Although its antiinflammatory activity is derived primarily by inhibition of COX [*e.g.*, 195], its full range of potentially chemopreventive activities are not yet known. For example, this drug is an antiproliferative (it inhibits ODC induction) [196], a tyrosine kinase inhibitor [43], and a free radical scavenger [43].

We sponsored a Phase I trial of ibuprofen in patients with previously resected colon adenomas (Dr. David S. Alberts, University of Arizona). A key objective of this study was to correlate suppression of rectal mucosal $PGE_2$ to inhibition of rectal mucosal cell proliferation.

*Indole-3-carbinol (I3C)* is a non-nutritive component of cruciferous vegetables [197]; consumption of these vegetables has been associated with decreased risk for cancer in humans [198]. I3C is an effective chemopreventive against a wide variety of carcinogens, presumably because of its capacity to induce both phase I and II enzymes involved in carcinogen metabolism [*e.g.*, 199–203]. I3C is very effective when administered before or during carcinogen administration [204,205] and has been shown to decrease carcinogen-DNA binding [*e.g.*, 205,206]. I3C both induces phase II enzymes [207] and several groups of cytochrome P450-dependent isozymes, including TCDD-type (CYP1A), phenobarbital-type

(CYP2B) and dexamethasone-type (CYP3A) [201,202,208]. These enzyme-inducing effects appear to be due to condensation products produced upon contact with gastric acid [209–211], and some of the products formed also demonstrate chemopreventive activity [204,212]. These multiple products with different enzyme inducing activities [211,213] may at least partially explain the pleiotropic effects of I3C.

Importantly, I3C's tumor inhibition in estrogen-responsive tissue [214,215] may result from increased estrogen conjugation and excretion via induction of phase II enzymes [216], as well as modulation of cytochrome P450-dependent estradiol metabolism. Increased estradiol-16α-hydroxylation has been associated with increased breast cancer risk in women [217] and mice [218], and 16α-hydroxyestrone has been reported to be genotoxic to mammary cells [219]. I3C increases estradiol 2-hydroxylation at the expense of 16α-hydroxylation under the same experimental conditions in which it reduces mammary [214] and endometrial [215] tumor development. The drug also enhances estradiol 2-hydroxylation in humans [220].

Besides mammary gland [204,208,214,221] and endometrium [215], I3C is chemopreventive in several animal cancer models including liver [*e.g.*, 222], tongue [223], lung [205], and forestomach [204]. Because of its inhibition of mammary gland carcinogenesis and its ability to induce estradiol 2-hydroxylation, breast is the target organ of highest clinical interest for I3C.

Thirty-day toxicity studies in rats and dogs have been completed; results of 90-day studies in rats and dogs are pending. A three-month Phase I trial in patients at high risk for breast cancer will be started soon.

*Perillyl alcohol* is a cyclic monoterpene occurring in numerous species of plants including mints (*Mentha piperita* and *M. spicata*), lavender, perilla (*Perilla frutescens*), *Cymbopogon*, citrus, and cranberries [224–226]. The monoterpene was given "Generally Recognized as Safe" (GRAS) status as a flavoring agent by FEMA in 1965, is approved as a food additive by the FDA and the Council of Europe [226], and is also used as a fragrance in perfumes, soaps, detergents, lotions, and creams.

The agent is a hydroxylated derivative of *d*-limonene. Both monoterpenes have demonstrated preclinical chemopreventive and chemotherapeutic activity possibly through metabolism to perillic and dihydroperillic acids [227,228]. These metabolites selectively inhibit isoprenylation of small (21–26 kDa) guanine nucleotide-binding proteins [229, 230], either by interaction with protein: prenyl transferases [231,232] or by selectively decreasing *ras* levels [233]. Some of these proteins are potentially oncogenic [229], such as the *ras* product p21. Perillyl alcohol also modulates the mevalonate-cholesterol pathway at other points distal to HMGCoA reductase, *i.e.*, synthesis of ubiquinone and conversion of lathosterol to cholesterol [234]. Inhibition of ubiquinone synthesis may slow proliferation of tumor cells, which often rely on glycolysis for ATP production. Other potentially chemopreventive activities attributed to perillyl alcohol include inhibition of tumor cell proliferation [234–237] (possibly through $G_1$ cell cycle block [238]), induction of differentiation [239], apoptosis [240], and enhancement of TGFβ growth regulatory activity [240–243]. The monoterpene also has the potential to induce cytochrome P450, GST and glucuronyl transferase activities, in analogy to other hydroxylated analogs of *d*-limonene [244].

Preclinical chemopreventive and chemotherapeutic [229,239,245] data are available for both perillyl alcohol and *d*-limonene. We found that perillyl alcohol at 2000 ppm in diet inhibited adenoma and adenocarcinoma development in AOM-induced rat colon (Dr. Bandaru S. Reddy, American Health Foundation, unpublished results). In published studies, perillyl alcohol also has been shown to inhibit rat liver [240] and hamster pancreatic [246] tumor development; in chemotherapeutic studies, it significantly reduced growth of established hamster pancreatic tumors [246], caused regression of established rat mammary gland tumors [247], and retarded growth of a prostate tumor cell xenograft in athymic nude mice [245]. The parent compound *d*-limonene has been shown to inhibit the growth of mouse lung [99] and skin tumors [248], rat mammary gland tumors induced by MNU, DMBA, and direct *in situ* transfer of v-Ha-*ras* [249–252], and rat liver tumorigenesis [244,253], as well as regress established mammary tumors [254,255]. However, *d*-limonene's clinical potential is limited by the large doses needed for efficacy [243,31]. For example, the minimum dietary dose for rat mammary tumor prevention is 1–5%, which is equivalent to 35–175 g daily for a 70 kg human. In comparison, perillyl alcohol is 5–10 times more potent in *in vitro* assays [229,230] and in

chemotherapy studies *in vivo* [247], suggesting that smaller doses might provide clinical benefit.

Besides the rat colon studied cited above, we are conducting preclinical efficacy studies of perillyl alcohol in three other models in which *ras* activation is a significant component of tumorigenesis—MNU-induced rat mammary gland, B(*a*)P-induced mouse lung, and BOP-induced hamster pancreas. Ninety-day toxicity studies in rats and dogs have also been completed. The results of these preclinical studies suggest that initial doses selected for clinical trials of 0.5–3.5 g/m$^2$ or 0.87–6.1 g/day are appropriate and well below the MTD of 28 g/day for men and 25.5 g/day for women extrapolated from the preclinical toxicity studies (*ca.* 400 mg/kg-bw/day or 2.6 mmol/kg-bw/day). After Phase I studies are completed, we plan to initiate short-term Phase II trials in breast and prostate.

*Phenethyl isothiocyanate (PEITC)* occurs naturally as its thioglucoside conjugate, gluconasturtiin, in many cruciferous vegetables including watercress, turnip, Chinese cabbage, cabbage, broccoli, cauliflower, horseradish, and canola oil [256–262]. This glucosinolate undergoes enzymatic hydrolysis whenever the raw wet plant material is crushed, thus releasing PEITC [ 259].

Chemopreventive effects of PEITC most likely result from its inhibition of specific cytochrome P450 enzymes (*e.g.*, CYP2E) which activate procarcinogens. For example, results from numerous animal efficacy studies have shown that PEITC inhibits lung tumor induction by the tobacco-specific nitrosamine NNK when it is administered during the initiation phase of carcinogenesis but not when administered post-initiation [263].

Besides NNK- and DMBA-induced lung tumors [263–267], PEITC has demonstrated chemopreventive efficacy in several other animal cancer models when administered prior to carcinogens requiring metabolic activation, including MBN-induced esophageal squamous papillomas [268–270], and DMBA- or B(*a*)P-induced forestomach in mice [ 271]. Thus, the primary efficacy of PEITC preferentially has been with nitrosamines which are activated by CYP2E.

Acute and subchronic safety studies have been carried out in F344 rats and a 14-day study has been done in female A/J mice. We are carrying out a 90-day safety study in dogs, prior to a Phase I trial in chronic smokers.

*9-cis-Retinoic acid* is a stereo- and photoisomer of all-*trans*-retinoic acid [272]. As described above, the cancer chemopreventive activities of the all-*trans* and 13-*cis* isomers of retinoic acid [24] are well recognized. Some retinoid effects are mediated by intracellular receptors which function as ligand-dependent transcription factors. These receptors are classified into two subfamilies, RARs and RXRs, based on differences in primary structure, sensitivity to synthetic retinoid ligands, and ability to regulate expression of different target genes [273]. all-*trans*-Retinoic acid binds directly to and transcriptionally activates RARs; however, although it can activate RXRs to regulate expression of target genes, it is not a ligand for this subfamily of receptors [272]. 9-*cis*-retinoic acid is a high affinity ligand for the RXRs [273,274], being up to 40 times more potent than all-*trans*-retinoic acid in transactivating RXRs. It also binds to and transactivates RARs, serving as a common "bifunctional" ligand [272].

RXRs can form homodimers and are capable of acting independently; however, they also form stable solution heterodimers with receptors for vitamin D and thyroid hormone, and with RARs. These interactions result in positive or negative regulation of transcription. The ability of RXRs to heterodimerize with receptors responsive to several ligands suggests a central role for RXRs in hormonal signaling. Thus, RXRs define a retinoid signaling pathway distinct from that mediated by the RARs [272]. This, together with the observation that the tissue distribution of RXRs and RARs differs [272], suggests that 9-*cis*-retinoic acid, as the high-affinity ligand for the RXRs, might have distinct chemopreventive properties.

9-*cis*-Retinoic acid can be delivered exogenously; however, it can also be formed from the all-*trans* isomer. It has been shown to be an *in vitro* and *in vivo* metabolite of all-*trans*-retinoic acid and has been identified as an endogenous component of mouse liver and kidney [273]. Interconversion could afford a novel method for differential, cell-specific regulation of the two retinoid signaling pathways [272].

This retinoid itself is a highly effective inhibitor of rat mammary tumorigenesis [274], and it also significantly enhances the efficacy of suboptimal doses of the antiestrogens tamoxifen [274] and raloxifene [275]. Possible activities contributing to its chemopreventive efficacy include proliferation [274,276] and angiogenesis [277] inhibition, and differentiation [274,278,279] and apoptosis [280,281] induction. In

mammary tissue, the mechanism may be down-regulation of the estrogen receptor [282] or inhibition of estrogen-responsive gene transcription [283], suggesting a reason for enhanced efficacy when administered with an antiestrogen. We are currently evaluating the preclinical efficacy of 9-*cis*-retinoic acid in rat mammary and prostate models and have found that it inhibits ACF in rat colon [53].

Limited published clinical data indicate that the oral MTD is 100–140 mg/m$^2$ (*ca.* 11.5 μmol/kg-bw/day) for four weeks in cancer patients; headache was the main dose-limiting toxicity [284–287]. Other common adverse effects were hyperlipidemia, dry skin and myalgia/arthralgia. As in rodents, 9-*cis*-retinoic acid appears to be absorbed ($t_{max} \leq 2$ hr) and excreted ($t^1/_2 \approx 1.6$ hr) fairly rapidly. Significant amounts of metabolites were excreted in urine; high amounts of presumably unabsorbed parent compound were found in feces. It is possible, but not yet established that, like all-*trans*-retinoic acid, the 9-*cis* isomer induces its own metabolism in humans. We are planning short-term Phase II clinical trials in breast cancer and CIN II/III patients; studies in head and neck and prostate are also under consideration. Based on the preclinical efficacy of the combination of this retinoid with tamoxifen and with raloxifene in MNU-induced rat mammary gland, combinations with an antiestrogen in the breast cohort may also be clinically useful.

*Sulindac sulfone* is one of two major metabolites of the NSAID sulindac. Like its parent, the sulfone has potential for inhibiting colon carcinogenesis. It inhibited induction of AOM-induced adenomas and adenocarcinomas in rat colon [288], and has also inhibited growth of at least three human colon cancer cell lines, a human colon polyp cell line, and a human polyp explant system [196]. Also, it inhibited MNU-induced rat mammary gland tumors [289]. Unlike sulindac, the sulfone is not an antiinflammatory drug and does not significantly inhibit COX [289]. Since the sulfone does not inhibit prostaglandin synthesis, it should not display the pronounced gastrointestinal toxicity associated with NSAIDs.

The chemopreventive mechanism of action of sulindac sulfone is currently being investigated. It does not appear to act by directly inhibiting cell proliferation [290], rather its growth inhibitory activity may be attributable to increased apoptosis [291].

We are now conducting a Phase Ib multidose safety and pharmacokinetics trial of sulindac sulfone in FAP patients at oral doses of 200, 300 and 400 mg bid for six months (Dr. G. Thomas Budd, Cleveland Clinic Foundation). As for sulindac, the major clinical target for sulindac sulfone will be patients with previous colorectal adenomas, and efficacy studies in this target population will be carried out first in FAP patients with partial colectomies. Since the total population of FAP patients with partial colectomies and, hence, suitable for chemopreventive intervention, is small, an attempt will be made to get preliminary efficacy information from this Phase I study as well as safety and pharmacokinetics data. The efficacy data will be dose-range finding based on the effects of sulfone treatment on regression and number of polyps. Should this Phase Ib study show low toxicity and demonstrate drug effect on adenomatous colon polyps, Phase II efficacy studies in FAP patients will be undertaken.

*Tea/epigallocatechin gallate (EGCG).* Although not conclusive, epidemiological studies have suggested a protective effect of black or green tea consumption against human cancers [292] such as breast [293], colon and rectum [294], gall bladder [295], liver [293], lung [293, 296], nasopharynx [297], pancreas [298], stomach [294,297], and uterus [293].

Tea compounds have demonstrated chemopreventive activities in animal cancer models in colon [299] and large intestine (EGCG) [300], duodenum (EGCG) [301, 302], esophagus [303,304], forestomach [305–309], liver [310,302], lung [305–309, 311–316], mammary gland [317,318], and skin [302,309,319]. We are also carrying out studies in colon, bladder, and esophagus with black and green tea extracts.

Multiple mechanisms [132,294,319–321] may be responsible for the chemopreventive properties of tea, including inhibition of lipid peroxidation and free radical formation (antioxidant activity), ODC, lipoxygenase and COX, and protein kinase C and cellular proliferation. Tea compounds also inhibit carcinogen-DNA binding and adduct formation by modulation of cytochrome P450 enzymes and enhancement of Phase II enzymes such as GST, as well as GSH-Px, catalase, and NAD(P)H:quinone reductase, and they enhance gap junction intracellular communication. Additionally, EGCG has a so-called "sealing effect" [322]; it inhibits interaction of tumor promoters, hormones, and various growth factors with their receptors.

We are now conducting preclinical 28-day and

90-day toxicology studies in rats and dogs for black and green tea polyphenols and EGCG. A major aspect in the further development of tea is characterization of standardized extracts for testing. Such standardization is being attempted in the preclinical efficacy and toxicity studies we are now carrying out.

*Ursodiol* is a minor bile acid found in trace amounts in human and rat bile. Some bile acids, such as deoxycholic acid (DCA), lithocholic acid (LCA), cholic acid (CHOL), and chenodeoxycholic acid (CDCA), have been implicated in colon tumor promotion due to cytotoxicity and compensatory cellular proliferation [323]. Particularly, the blood concentration of DCA in humans has been related to the incidence of adenomatous polyps [323]. In contrast, ursodiol appears to neutralize the harmful effects of other bile acids [324] by inhibiting 7α-dehydroxylase in colonic bacteria, resulting in significantly lower production of DCA from the primary bile acids CHOL and CDCA [325]. *In vitro* studies with erythrocytes and hepatocytes demonstrated that ursodiol also stabilizes cell membranes from the cytotoxic effects of DCA and CDCA [324].

A single *in vivo* chemoprevention study with ursodiol has been reported in which the incidence of tumors in the AOM-induced colonic carcinogenesis model was significantly reduced in rats administered 0.4% ursodiol in their diet (*ca.* 200 mg/kg-bw/day)[326].

Preclinical toxicity data generated for FDA approval of ursodiol (Actigall®) as a treatment for the dissolution of gallstones included two-year carcinogenicity studies in CD-1 mice and Sprague-Dawley rats and reproductive studies in rats and rabbits [327]. The only toxic effect was a significantly increased incidence of pheochromocytomas in the adrenal medullas of male and female rats, a condition which is common in that species. Adverse reactions to Actigall® are either gastrointestinal or dermatological, usually minor and infrequent [327]. The most common is diarrhea which occurred in <1–27% of subjects exposed to recommended doses of 8–10 mg/kg-bw qd [323]. We are sponsoring a Phase I study in patients with previous colon adenomas and cancers (Drs. David S. Alberts and David Earnest, University of Arizona). These investigators have also gathered preliminary clinical efficacy data.

*Vitamin $D_3$ analogs.* The inverse association between vitamin D and cancer was derived from the geographical variation in prostate, breast, and colon cancer death rates, which increase with increasing latitude and decreasing sunlight intensity [328–333]. Subsequent epidemiological studies suggested inverse relationships between dietary vitamin D intake or serum 25-hydroxyvitamin D3 levels and colon cancer risk [334–340]. Vitamin D3 is metabolized in the liver to 25-hydroxyvitamin D3 ($t_{1/2}$ in weeks), and the kidney converts this compound to the physiologically active hormone 1α,25-dihydroxyvitamin $D_3$ [341]. The activated vitamin binds to the intracellular vitamin D receptor, heterodimerizes with RXR receptor, and binds DNA. Protein transcription is initiated in the appropriate cell types, producing the classic net increase in circulating calcium and phosphorus by increasing intestinal absorption and mobilization from bone, and decreasing urinary excretion [342,343]. Receptors are also found in tissues unrelated to these functions, such as mammary gland, colon, prostate, and skin [344,345]. Although the function of the receptors is unknown, vitamin D has chemoprevention-related activities in these and other tissues, including inhibition of proliferation and DNA synthesis [346–348], modulation of signal transduction by calcium and protein kinase C [349], modulation of c-*myc*, c-*fos* and c-*jun* oncogene expression [350–353,], inhibition of ODC induction [354], lipid peroxidation [355] and angiogenesis [349], and induction of differentiation [356–359], TGFβ expression [349,360,361] and, possibly, apoptosis [362]. Unfortunately, the concentrations of 1α,25-dihydroxyvitamin $D_3$ necessary to suppress cell growth in culture would produce severe hypercalcemia *in vivo* [363]. This has led to the development of vitamin $D_3$ analogs (deltanoids) which retain the chemopreventive activities of the hormone with less calcium toxicity; they appear to have decreased affinity for the vitamin D receptor, although some have greater potency as inducers of transcription *in vitro* [364]. The analogs which we are considering for further development as cancer chemopreventive drugs are Ro 23-7553, Ro 24-5531, and Ro 24-2637.

*In vitro* studies first demonstrated that 1α,25-dihydroxyvitamin $D_3$ inhibited proliferation and induced differentiation in human leukemia cell lines [352, 365,366] and cells from human prostate [367], skin [368], breast [347], and colon cancers [346,369,370]; the analogs were found more potent in leukemia cell lines [*e.g.*, 371]. We found that the vitamin and all the analogs reduced carcinogen-induced morphologic transformation of mouse mammary organ

cultures and rat tracheal epithelial cells. *In vivo*, vitamin $D_3$ inhibited rat bladder and colon carcinogenesis; 1α,25-dihydroxyvitamin $D_3$ was effective against rat colon and mouse skin cancer models [372–375]. Only the most potent analog—Ro 24-5531—has been tested in a carcinogen-induced animal model; it inhibited rat mammary gland carcinoma development [376]. Inhibition was enhanced by treatment in combination with tamoxifen [377]. We are currently testing the deltanoids Ro 23-7553 and Ro 24-5531 in rat mammary gland and colon carcinogenesis models, and we will undertake preclinical toxicity studies of the most potent analogs. Published information demonstrates that 1α,25-dihydroxyvitamin $D_3$ produces significant toxicity in rodent studies and in humans, primarily hypercalcemia, weight loss and tissue calcification [378]. Some evidence for embryotoxicity and teratogenicity was also found in animal studies; however, maternal toxicity was often a contributing factor. The analogs of vitamin $D_3$ were synthesized to reduce toxicity while retaining chemopreventive efficacy. The analogs appear to have less potential for calcium-related adverse effects in both chicken and rodent models [378].

p-*Xylylselenocyanate (p-XSC).* Selenium deficiency has been linked epidemiologically to significantly increased incidences or mortality from cancers in bladder, breast, gastrointestinal tract, lung, and prostate, although these relationships are inconsistent between studies and geographical areas [379– 386]. The most consistent relationships have been seen in prospective studies in males (*e.g.*, gastrointestinal tract, pancreas and all sites). High doses of both inorganic and organic selenium compounds have inhibited chemically and virally induced tumors in animal mammary gland, colon, skin, lung, trachea, liver, stomach and pancreas models, as well as the development of transplanted tumors [387]. However, selenium can be toxic. High chronic intake in animals causes body weight loss, liver damage, splenomegaly, pancreatic enlargement, anemia, hair loss, and abnormal nails. In humans, the estimated MTD is 500 μg organic Se/day (0.09 μmol/kg-bw/day); ten times this dose of organoselenium compounds causes garlic breath, nausea, fatigue, irritability, dermatitis, hair loss, and nail changes or loss [388].

Since the major dietary source of selenium is in organic forms (primarily selenomethionine, selenomethylselenomethionine, selenocystine, and selenocysteine) from grains, meat, yeast, and certain vegetables [389], organoselenium compounds would appear to be the most appropriate forms to investigate for chemopreventive potential. However, the effective and toxic doses are close, especially for those compounds containing amino acids (*e.g.*, selenocysteine and selenomethionine) which are nonspecifically incorporated into proteins. When incorporated into proteins, the selenized amino acids do not release the monomethylated selenium metabolites, replete GSH-Px, or participate in other reactions which have been associated with chemopreventive activity [388,390]. A search for more effective, less toxic organoselenium agents has led to the synthesis of *p*-XSC and benzylselenocyanate (BSC). *p*-XSC has a chemopreventive index (defined as the ratio of MTD to effective dose) of 6.5 in rats, compared with indices of 1, 1.3, 1.3, 2.0, and 2.5 for selenomethionine, sodium selenite, potassium selenocyanate, methylselenocyanate and BSC, respectively [391,392]. Unlike *l*-selenomethionine, it is not incorporated into proteins; however, in rats *p*-XSC has some liver and hematopoietic system toxicity [391].

In published preclinical studies, *p*-XSC was effective in the AOM-induced rat colon [393], the DMBA-induced rat mammary [392], and the NNK mouse lung [394] carcinogenesis models during initiation and/or postinitiation phases. The chemopreventive effects of *p*-XSC have been attributed to several mechanisms, including its antioxidant and antiinflammatory activity [391–393,395,396], UDP-glucuronyl transferase induction (*i.e.*, increasing carcinogen detoxification) [397], apoptosis induction [398, 399], and stimulation of differentiation by activating thyroid hormone [400].

We are currently evaluating *p*-XSC in 30- and 90-day preclinical toxicity studies in dogs (and may do a second 90-day study in rats), preparatory to Phase I clinical studies. Based on the preclinical efficacy data, if the animal toxicity and Phase I studies are completed satisfactorily, Phase II trial(s) would be initiated in high-risk breast and colon cancer cohorts, or smokers at high risk for lung cancer.

## FUTURE CHEMOPREVENTIVE AGENT DEVELOPMENT

Other agents and classes of agents are nearing clinical development as chemopreventives. Primary examples are aromatase inhibitors which have promise in breast, and possibly, prostate. The nonsteroidal

aromatase inhibitor (+)-vorozole was a potent inhibitor of rat mammary gland cancer in studies in our drug development program [401]. Several aromatase inhibitors, including other nonsteroidals such as anastrazole (Arimidex®) and steroidal compounds such as exemestane, are already being developed by pharmaceutical companies for breast cancer treatment in postmenopausal women. In fact, anastrozole is already approved by the FDA for treatment of postmenopausal breast cancer patients who have failed tamoxifen therapy. Because of this previous *clinical* testing, such aromatase inhibitors do not need further Phase I testing, and may be evaluated directly in Phase II chemoprevention studies. We are particularly interested in the prospects for an aromatase inhibitor in a short-term Phase II breast chemoprevention study.

Protease inhibitors have several chemoprevention-related activities. They depress error-prone repair in bacteria, oncogene expression, and basement membrane degradation [402]. Bowman-Birk soybean trypsin inhibitor is a protease inhibitor demonstrating chemopreventive activity and nearing clinical development [403]. A number of carotenoids have been described which may be more potent than β-carotene. Examples are α-carotene, lutein, and lycopene. α-Carotene has only half the provitamin A activity of β-carotene, but is ten times more efficacious than β-carotene in preventing lung, liver, and skin carcinogenesis in animal models [404].

Both vitamin E [405] and *l*-selenomethionine (Dr. Larry Clark, University of Arizona, unpublished results) appear to show activity against prostate cancer. The rationale for chemopreventive activity of selenium compounds was described above. *l*-Selenomethionine is active against mammary cancer [406– 410]. Because both agents have been well-characterized in previous clinical trials and dietary studies, it is possible to test them in Phase II trials without further Phase I testing. We are particularly interested in evaluating a vitamin E and selenium compound combination in breast and prostate.

The number and promise of the agents described indicate that continued research and development will result in efficacious and practical chemopreventive drugs. As knowledge in molecular biology and the basic cellular processes in carcinogenesis increases, more chemopreventive agents that are directed to repair or suppress early genetic lesions and control cellular growth mechanisms (*e.g.*, apoptosis, angiogenesis, signal transduction pathways) may be identified. A major aspect of future chemopreventive drug development will be the mechanism-directed identification and testing of new agents. Both the NCI Chemoprevention Branch and other investigators are following this mechanism-based approach. For example, interest in developing specific inhibitors of COX-2 was described above, and we have recently described an approach to the development of inhibitors of EGFR-mediated signal transduction [411].

Very interesting examples of the potential application of specific mechanistic considerations to the design of chemopreventive agents are inhibitors of specific steps in signal transduction pathways. *ras* inhibitors which mimic the C-terminal peptide sequence of *ras* oncogene products [*e.g.*,412,413]. A recent publication demonstrates the cancer inhibitory activity of one of these compounds, L-744,832, which causes regression of mammary and salivary gland tumors in *ras* transgenic mice [413].

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