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Preclinical Efficacy Evaluation of Potential Chemopreventive Agents in Animal Carcinogenesis Models: Methods and Results From the NCI Chemoprevention Drug Development Program

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Abstract In the NCI, Chemoprevention Branch drug development program, potential chemopreventive agents are evaluated for efficacy against chemical carcinogen-induced tumors in animal models. This paper summarizes the results of 144 agents in 352 tests using various animal efficacy models. Of these results, 146 were positive, representing 85 different agents.

The target organs selected for the animals model are representative of high-incidence human cancers. The assays include inhibition of tumors induced by MNU in hamster trachea, DEN in hamster lung, AOM in rat colon (including inhibition of AOM-induced aberrant crypts), MAM in mouse colon, DMBA and MNU in rat mammary glands, DMBA promoted by TPA in mouse skin, and OH-BBN in mouse bladder.

The agents tested may be classified into various pharmacological and chemical structural categories that are relevant to their chemopreventive potential. These categories include antiestrogens, antiinflammatories (*e.g.*, NSAIDs), antioxidants, arachidonic acid metabolism inhibitors, GST and GSH enhancers, ODC inhibitors, protein kinase C inhibitors, retinoids and carotenoids, organosulfur compounds, calcium compounds, vitamin D_3 and analogs, and phenolic compounds (*e.g.*, flavonoids). The various categories of compounds have different spectra of efficacy in animal models. In hamster lung, GSH-enhancing agents and antioxidants appear to have high potential for inhibiting carcinogenesis. In the colon, NSAIDs and other antiinflammatory agents appear particularly promising. Likewise, NSAIDs are very active in mouse bladder. In rat mammary glands, retinoids and antiestrogens (as would be expected) are efficacious. Several of the chemicals evaluated also appear to be promising chemopreventive agents based on their activity in several of the animal models. Particularly, the ODC inhibitor DFMO was active in the colon, mammary glands, and bladder models, while the dithiolthione, oltipraz, was efficacious in all the models listed above (*i.e.*, lung, colon, mammary glands, skin, and bladder).

Key words: Animal models, carcinogenesis, chemoprevention, drug development

A major objective of the NCI, Chemoprevention Branch is to identify and develop chemopreventive chemicals as drugs for human use. Agents tested in the program are selected from several sources. To date, the majority of candidates have been identified by review of the literature in basic and epidemiological sciences related to chemoprevention [1,2] and from studies of classes of compounds with chemical structural or pharmacological properties related to known chemopreventive agents. A systematic methodology which considers chemopreventive efficacy, toxicity, pharmacokinetics, potential for clinical use, commercial availability, source, and cost of the agent is applied to identify and rank candidates [3]. Other sources, including collaborations with academia and pharmaceutical companies, are becoming increasingly important. The candidate agents are then screened via a hierarchial network of preclinical efficacy tests including *in vitro* mechanistic assays, *in vitro* efficacy screens, and animal cancer efficacy studies. Based on the results generated by these assays as well as preclinical toxicology and pharmacokinetic studies, the promising agents progress to clinical trials [4–6]. Overviews of this program and preliminary test results have been published previously [3,4,7–10].

The subject of this paper is the results of chemopreventive efficacy testing in animal cancer models carried out in the Chemoprevention Branch program. The target organs selected for the models are representative of high-incidence human cancers. The assays include inhibition of tumors induced by MNU[1] in hamster trachea, DEN in hamster lung, AOM in rat colon (besides tumors, inhibition of AOM-induced aberrant crypts, a putative intermediate biomarker for colon cancer is evaluated), MAM in mouse colon, DMBA and MNU in rat mammary gland, DMBA promoted by TPA in mouse skin, and OH-BBN in mouse bladder. Besides identification and evaluation of promising chemopreventive agents, another objective is the characterization and validation of these assays and other appropriate models. To this end, new models are considered continually, and a portion of the testing carried out is of well-established chemopreventive agents to characterize test parameters such as appropriate carcinogen dose, treatment time, and effects of diet.

Initially, animal efficacy models are used to determine the overall efficacy (positive or no effect) of the candidate agents. For the most promising agents, additional testing in animal models is carried out to establish dose-response relationships, evaluate combinations of agents, determine organ specificity and elucidate mechanisms of cancer prevention. Various formulations and doses of the agent or cancer-susceptible subpopulations of animals (*e.g.*, aging animals) may be used.

Selection of models for testing an agent *in vivo* is based on several factors including the efficacy of the agent in *in vitro* screens; the known pharmacology of the agent (*e.g.*, compounds such as tamoxifen with antiestrogenic activity may be evaluated in the rat mammary model); published data on the agent's *in vivo* efficacy and mechanism of action; and commercial availability of the agent (*e.g.*, agents in limited supply may be selected for testing in the aberrant crypt foci assay; the five-week assay requires a relatively small amount of agent).

METHODS

In animal chemopreventive efficacy models, a carcinogen is administered at a high enough dose level to induce a significant incidence of tumors in the target tissue. The appropriate carcinogen dose and treatment schedule are selected to insure that the efficacy of the potential chemopreventive agent can be evaluated accurately (*e.g.*, not masked by carcinogen toxicity). The test agent is usually administered in the diet unless there are problems with its stability or absorption (*e.g.*, β-carotene is poorly absorbed in rats on oral administration) at 0.4 and 0.8 MTD or up to 2% in diet; the MTD for each agent is established from the dose range-finding studies. For the chemoprevention studies, the MTD is defined as the highest dose which does not cause ≥10% reduction in weight gain or final weight after six weeks of administration. Most of the results described in this paper are from tests in which the agents were administered from one week prior to the initial carcinogen dose to the end of the study—*i.e.*, before, concurrently, and after administration of the carcinogen (schedule C). In some studies, other treatment schedules were used.

[1]Please see Appendix A: Abbreviations at the end of this Supplement.

These include administration of the agent only before or during (schedule A) or after (schedule B) treatment with carcinogen.

Efficacy is measured as the percentage by which the agent reduces the tumor incidence or multiplicity, or increases the latency of tumor appearance, compared with carcinogen-treated controls. Both gross pathology and histopathological analysis are used to determine these parameters. As noted below, effects on tumor incidence are measured in each model, while those on multiplicity and latency are measured only when possible (*e.g.*, in rat mammary glands).

To be classified as having a cancer inhibitory effect, the agent must cause a statistically significant ($p<0.05$) decrease in tumor incidence or multiplicity, or a statistically significant increase in tumor latency, compared with carcinogen-treated controls. Tumor latency is measured as the mean time to appearance of the first tumor or the time to 50% tumor incidence. Appropriate standardized statistical analyses have been established for evaluating each model [*e.g.*, 11].

The adequacy of the experiment is also evaluated regardless of the magnitude of the inhibitory effect observed. The criteria for adequacy include: (1) the number of animals in each treatment or control group was sufficient to demonstrate a statistically significant inhibitory effect of the agent, (2) evidence of carcinogenicity in concurrent carcinogen-treated control animals was established under the conditions of the experiment, (3) the survival of animals in agent-treated and carcinogen-treated control groups was adequate to allow proper statistical analysis, (4) toxicities observed due to carcinogen and/or agent treatment were not so severe as to confound the results of the experiment, and (5) significant weight loss in the agent-treated groups compared with the carcinogen-treated control group was not observed. This is an important consideration since decreased weight gain *per se* is known to reduce tumor response under certain experimental conditions [12,13].

Description of Animal Efficacy Models

Hamster Respiratory Tract Two hamster models have been used in the Chemoprevention Branch program to evaluate chemopreventive efficacy against respiratory tract cancers—MNU-induced tracheal squamous cell carcinomas and DEN-induced lung adenocarcinomas.

In the MNU model, 5% MNU in saline is administered once a week for 15 weeks by a specially designed catheter which exposes a defined area of the trachea of male Syrian golden hamsters to the carcinogen [14,15]. Test agents are administered in the diet according to schedule C, starting one week before the first carcinogen administration and continuing to the end of the experiment (180 days from the first carcinogen treatment). Administration of the MNU for 15 weeks typically produces tumors in 40–50% of treated animals within six months. Efficacy is measured as percent reduction of tumor incidence compared with carcinogen controls.

In the DEN model [15,16], twice weekly subcutaneous injections of 17.8 mg DEN/kg-bw for 20 weeks starting at age 7–8 weeks usually produce tracheal tumors in 90–100%, and lung tumors in 40–50%, of treated male Syrian hamsters. Serial sacrifice studies have shown that the lung tumors originate from pulmonary Clara and endocrine cells while the tracheal tumors are derived from the basal cells of the respiratory epithelium [16]. This model may be especially appropriate for examining the chemopreventive activity of chemical agents, since lung tumors with features of pulmonary endocrine cells (small cell cancer) account for approximately 30% of all human lung cancers [15]. The agent treatment schedule and evaluation of efficacy is the same as for the MNU-trachea model; efficacy is based primarily on percent reduction in lung tumor incidence.

Rat and Mouse Colon Intraperitoneal DMH injection produces colorectal adenocarcinomas in both rats and mice [8,17]. *In vivo*, DMH is first activated to AOM and then to the ultimate carcinogen, MAM. In the rat model, a single subcutaneous dose of 30 mg AOM/kg-bw given to seven-week old F344 male rats produces colon adenomas and adenocarcinomas within 40 weeks; the total tumor incidence is approximately 70% [17]. In the mouse model, 9–11-week old female CF_1 mice are injected intraperitoneally with 20 mg MAM acetate/kg-bw, 1x/week, for four or six weeks. Colon tumors appear within 38 weeks after dosing [18]. In both models, efficacy is based on percent reductions in tumor incidence, multiplicity, or both compared with the carcinogen-treated controls.

Aberrant crypt foci are single and multiple colonic crypts containing cells exhibiting dysplasia [19,20]. These aberrant crypt foci are putative precancerous lesions and are being evaluated as intermediate biomarkers for colon cancer in rodents. In the assays cited here, two protocol variations have been used. In all studies, male F344 rats received the chemopreventive agent at 0.4 or 0.8 MTD in

semi-purified diet (AIN-76A) and two injections of 15 mg AOM/kg-bw one week apart or one injection of 30 mg AOM/kg-bw. The most frequent treatment schedule (A) primarily detects agents which inhibit initiation of colon crypts. The seven-week old rats received chemopreventive agent starting one week prior to the carcinogen. Treatment with the agent was continued for another four weeks until the end of the study. The second schedule (B) was designed to test effects of the chemopreventive agent on the post-initiation phase of colon carcinogenesis. Rats received the chemopreventive agent for four weeks, starting four weeks after the first carcinogen dose. At the end of treatment, the animals were sacrificed and the frequencies of aberrant crypt foci were determined by histopathologic evaluation (larger size and increased stain uptake, increased distance from luminal to basal surfaces of crypt cells, and enlarged pericyptal zone compared with normal crypts) after staining with methylene blue [20,21]. Using the five-week protocol, Wargovich [21] found a frequency of 80–140 aberrant crypt foci per colon in AOM-treated controls; Pereira [20] found approximately twice the number of aberrant crypt foci in animals treated in the morning (205.7 ± 16.0) than in the afternoon (110.2 ± 12.9, $p<0.01$).

Rat Mammary Glands Both MNU- and DMBA-induced mammary gland carcinogenesis models are used for efficacy testing. In the MNU model [14,22–25], a single intravenous injection of 50 mg/kg-bw (pH 5.0) is given to Sprague-Dawley rats, usually at 50 days of age. In some of the testing, the carcinogen has been administered to 120-day old animals, which may better model the human target population of mature adults [*e.g.*, 26]. In the DMBA model, female Sprague-Dawley rats are usually given single intragastric injections of 12 mg carcinogen at 50 days of age. This dose can produce a 80–100% incidence of total mammary tumors (adenocarcinoma, adenoma, and fibroadenoma) within 120 days post-carcinogen [14]. DMBA is activated in the liver, so testing with DMBA can detect agents which inhibit carcinogen activation (*e.g.*, those inhibiting cytochromes P-450). MNU produces 75–95% incidence of tumors within 180 days post-carcinogen. MNU does not require metabolic activation, and this model does not detect inhibition of carcinogen activation. However, MNU-induced tumors better model human breast cancer. Unlike DMBA-induced tumors, MNU-induced tumors are invasive (DMBA-induced tumors are encapsulated) and are predominantly adenocarcinomas (DMBA induces a high percentage of adenomas and fibroadenomas) [12]. Tumors induced by both carcinogens, like human breast cancers, are mostly hormone-dependent. Additionally, the tumors are associated with activated *ras*, which may be a factor in human breast cancer. In both models, chemopreventive activity is measured as percent reduction in adenocarcinoma incidence, multiplicity, and/or percent increase in adenocarcinoma latency compared with carcinogen-treated controls. Tumor multiplicity is typically in the range 3–4.4 in DMBA-treated controls and 2–4 in MNU-treated controls. Tumor latency is about 65–80 days in both models.

Mouse Skin A typical protocol for this model has been recently published [27]. In this two-stage carcinogenesis model, DMBA and TPA are used to induce skin papillomas and squamous cell carcinomas in the SENCAR mouse, a special strain with enhanced sensitivity to skin tumor induction. The mice are administered a single dose of 2.5 μg DMBA in 0.2 ml acetone. Beginning one week later, the tumor promoter TPA is applied at a dose of 5–10 μg in 0.2 ml acetone twice weekly to the same area of the dorsal skin as DMBA. Papillomas begin to appear after 6–7 weeks of treatment with TPA, and the animals are observed weekly to monitor tumor development until study termination after 18 weeks [28]. Chemopreventive activity is measured as percent reduction in incidence and multiplicity of papillomas and carcinomas.

Mouse Bladder In this model, chemopreventive activity is assessed against OH-BBN-induced urinary bladder invasive transitional cell carcinomas (TCC) that morphologically resemble a human variant of advanced urinary bladder TCC [29]. Male BDF (C57BL/6 X DBS/2-F_1) mice are given eight weekly doses of 7.5 mg OH-BBN by intragastric instillation beginning at 50 days of age [29–31]. Chemopreventive activity is measured as percent reduction in incidence of TCC compared with carcinogen-treated controls; the incidence in carcinogen controls is typically approximately 40% within the 180-day study duration [*e.g.*, 32,33].

RESULTS

Table I summarizes 352 test results (both positive and no effect) on 144 agents obtained in the Chemoprevention Branch drug development program by June 30, 1994. Of these results, 146 were positive, representing 85 different agents. The majority of the results are from tests according to schedule C; however, a few significant positive

Table I. Chemoprevention Branch Drug Development Program: Animal Efficacy Studies Summary of Test Results on Single Agents (as of June 30, 1994)

	Lung[1]		Colon			Mammary		Skin	Bladder
	Hamster		Rat Crypts	Mouse	Rat	Rat		Mouse	Mouse
Agent	DEN[2]	MNU	AOM	MAM	AOM	DMBA	MNU	DMBA	OH-BBN
N-Acetyl-*l*-cysteine		+[3]	NE [20][4]		+ [69]		+		+
S-Allyl-*l*-cysteine	NE		+(A)[5] NE(B)				NE		
Am 80							NE		NE
Am 580							NE		NE
4-Aminobenzoic Acid			NE [20]						
Aminoglutethimide							+		
Anethole Trithione	NE		NE		+ [69]	+	NE		NE
d,l-Arginine Hydrochloride			+				NE	NE	
Ascorbyl Palmitate		NE	NE [21]	NE	+	NE	NE	NE	NE
Aspirin			NE(A) [20] +(B)		+ [61]		NE		NE
BASF-47851							+		
Benzyl Isothiocyanate			NE [21]	NE				+	NE
Bismuthiol I	+		NE [20]				NE		+
2-Bromo-α-ergocryptine			NE				+		
4-Bromophenacyl Bromide			NE						
Butylated Hydroxyanisole			+ [21]	+				NE	NE
Butyric Acid, Sodium Salt			+ [21]	NE					
Caffeic Acid			NE [20]						
CAI			+(A) NE(B)						
Calcium Carbonate			+ [20]						
Calcium Chloride			+ [20]	NE					
Calcium Phosphate, Dibasic			NE [20]						
Carbenoxolone		NE	NE [20]		+	NE	+		NE
Carnosine			NE [20]				NE		
β-Carotene	NE	NE [182]							NE

Table I. Chemoprevention Branch Drug Development Program: Animal Efficacy Studies Summary of Test Results on Single Agents (as of June 30, 1994) *(continued)*

Agent	Lung[1] Hamster DEN[2]	Lung[1] Hamster MNU	Colon Rat Crypts AOM	Colon Mouse MAM	Colon Rat AOM	Mammary Rat DMBA	Mammary Rat MNU	Skin Mouse DMBA	Bladder Mouse OH-BBN
β-Carotene (Injectable)	NE [34]	NE [182]					+		
(+)-Catechin			NE [21]	NE		+	NE		NE
Ch 55							NE		NE
Chlorogenic Acid			NE [20]				NE		
Chlorophyllin, Copper Sodium			NE [20]				NE		
Chlorpheniramine Maleate			+						
Corn Oil			NE						
Cromolyn Sodium		NA	NE [21]	NE					
Curcumin			+ [20]	+			+		NE
DFMO	NE [34]	NE [100]	+(B) [21]	+	+ [62,63]	+ [100]	+		+ [32,33,179]
DHEA			NE [21]	+			+ [114]	NE	NE
DHEA Analog 8354			NE [20]		+ [63]		+ [114]		
Diallyl Disulfide		+	NE [20]		+ [69]		NE		NE
Diallyl Sulfide			+ [21]	NE			NE		NE
Diallyl Trisulfide							NE		
Dimethyl Fumarate			+ [20]		NE		NE		
Diphenhydramine		NE	NE [20]						
Dipyridamole							NE		
Ellagic Acid			NE [21]		+ [63]	NE	NE		NE
Esculetin							NE		NE
Ethylvanillin		+	NE				+		
Etoperidone			+				NE		
Farnesol			+						
Ferulic Acid			+						
Fish Oil (Menhaden)			NE [20]						
Fish Oil (NOS)							+		
Fluocinolone Acetonide			NE [21]	+					NE

Table I. Chemoprevention Branch Drug Development Program: Animal Efficacy Studies Summary of Test Results on Single Agents (as of June 30, 1994) *(continued)*

Agent	Lung[1] Hamster DEN[2]	Lung[1] Hamster MNU	Colon Rat Crypts AOM	Colon Mouse MAM	Colon Rat AOM	Mammary Rat DMBA	Mammary Rat MNU	Skin Mouse DMBA	Bladder Mouse OH-BBN
Folic Acid			NE [21]	NE			NE		NE
Fumaric Acid		+	+ [20]			NE	+		NE
Genistein			+ [20]						
Glucaric Acid							NE		
Glucaric Acid, Calcium Salt			+(A,B) [20,21]	NE			+		
Glucaric Acid, Potassium Salt			NE [21]	NE					
d-Glucaro-1,4-lactone							NE		
18β-Glycyrrhetinic Acid			+ [21]	+	NE [64]		+	+	NE
Hexamethylene Bisacetamide									NE
4-HPR	+ [15,34]	NE [182]	+(B)	NE		+	+ [24,115, 116]	+	NE [32, 33,179]
4-Hydroxyandrost-4-ene-3, 17-dione							NE [118]		
Ibuprofen		NE	NE(A) [20] +(B) [21]		+ [64]		+		+
Indole-3-carbinol		NE	+ [21]	+		+	+	NE	NE
Indomethacin			+ [21]	NE			NE		+ [180]
Inositol Hexaphosphate, Dodecasodium Salt						NE			
Inositol Hexaphosphate, Sodium			NE [21]	NE			NE		
Ketoprofen			+(A,B) [21]		+ [64]		NE		+
Lactic Acid, Calcium Salt			+						
Levamisole			NE [21]	NE					
d-Limonene			+				NE		
d-Mannitol			NE [21]	NE			NE		
Meclizine			+						
Meclofenamate, Sodium Salt			NE						

Table I. Chemoprevention Branch Drug Development Program: Animal Efficacy Studies Summary of Test Results on Single Agents (as of June 30, 1994) *(continued)*

Agent	Lung[1] Hamster DEN[2]	Lung[1] Hamster MNU	Colon Rat Crypts AOM	Colon Mouse MAM	Colon Rat AOM	Mammary Rat DMBA	Mammary Rat MNU	Skin Mouse DMBA	Bladder Mouse OH-BBN
Melatonin							NE		
2-Mercaptoethanesulfonate, Sodium Salt			NE [21]	NE			NE		+
2-Mercaptoethylamine Hydrochloride							NE		
d,l-Methionine			+						
4-Methoxyphenol			NE		NE		NE		
Methylene Blue			+				NE		
Molybdate, Sodium		NE	NE [21]	NE			+	NE	+
Morin							NE		
Nicotinic Acid			NE						
Nordihydroguaiaretic Acid			NE [21]				NE		
Oltipraz	+ [34]	+	+ [21]	+	+ [65,66]	+	+	+	+ [32,179]
l-2-Oxothiazolidine-4-carboxylate		NE					NE		
d,l-Palmitoylcarnitine		NE				NE	NE		
Phenethyl Isothiocyanate			NE [21]			NE	NE		
Phenhexyl Isothiocyanate			+		NE				
Piroxicam			+(A,B) [20,21]		+ [65–68]		NE	+	+ [32,179]
Polyvinylpyrrolidone			+						
Praziquantel							+		
Progesterone							+		
Propylene Glycol							NE		
Propyl Gallate			NE [21]	NE			NE	+(B)	
Purpurin			+ [21]	+			NE		
Quercetin			NE(A) [21] +(B)	+			NE		NE

Table I. Chemoprevention Branch Drug Development Program: Animal Efficacy Studies Summary of Test Results on Single Agents (as of June 30, 1994) *(continued)*

Agent	Lung[1] Hamster DEN[2]	Lung[1] Hamster MNU	Colon Rat Crypts AOM	Colon Mouse MAM	Colon Rat AOM	Mammary Rat DMBA	Mammary Rat MNU	Skin Mouse DMBA	Bladder Mouse OH-BBN
Quinacrine Hydrochloride			NE				NE		
13-*cis*-Retinoic Acid			+(B)						
9-*cis*-Retinoic Acid			+(B)						
all-*trans*-Retinoic Acid			+(B)						
Riboflavin 5'-Phosphate							NE		
Ro 16-9100			+(B)				+		NE
Ro 19-2968							+		
Ro 21-5535			+						
Ro 23-7553			+						
Ro 24-2637			NE						
Ro 24-5531			+						
RU 16117							+ [117]		
Rutin			NE(A) [20] +(B) [21]	+			NE		NE
Sarcophytol A							NE		
Selenate, Sodium				NE					
Selenite, Sodium	+ [182]	NE [182]	+ [21]	NE		+		NE	NE
l-Selenomethionine				NE					
Silymarin			NE [21]	NE			NE		NE
Simethicone			+ [20]						
β-Sitosterol			NE [21]	NE			NE		NE
Stevioside			+						
Sulfasalazine			NE [21]		NE				
Sulindac			+ [20]		+		NE		+
Suramin			+ [20]				NE		
Tamoxifen							+[115, 117]		

Table I. Chemoprevention Branch Drug Development Program: Animal Efficacy Studies Summary of Test Results on Single Agents (as of June 30, 1994) *(continued)*

Agent	Lung[1] Hamster DEN[2]	Lung[1] Hamster MNU	Colon Rat Crypts AOM	Colon Mouse MAM	Colon Rat AOM	Mammary Rat DMBA	Mammary Rat MNU	Skin Mouse DMBA	Bladder Mouse OH-BBN
Tamoxifen Citrate							+ [24, 117]		
Taurine			+		+ [69]		NE		
Temaroten							+		NE
Tetracycline			+						
Theophylline			+						
Thioctic Acid							NE		
Thiolutin							NE		
Thiosulfate, Sodium			+ [21]	NE					
2-Thioxo-4-thiazolidinone							NE		
Toremifene							+ [117]		
Triprolidine			+						
Uric Acid							NE		
Vanillin							NE		
Verapamil			+						
Vitamin A	NE		+(B)						
Vitamin D_3			NE [21]	NE					
Vitamin E Acetate		NE [182]	NE				NE		NE
Vitamin E Succinate PEG 1000		NE					NE		NE
Vitamin K_3							NE		
(+)-Vorozole							+ [118]		

[1] Each column contains the test results for a single animal model. The column header for each model describes the cancer target (lung, colon, mammary glands, skin, or bladder), animal species (hamster, rat, or mouse), and carcinogen used to induce tumors (DEN, MNU, AOM, MAM, DMBA, or OH-BBN). These models are described in the text of this paper.

[2] Please see Appendix A: Abbreviations at end of this Supplement.

[3] The test results are as follows: +, the agent induced a statistically significant decrease in tumor incidence or tumor multiplicity, or increase in tumor latency compared with the carcinogen-treated controls at one or more dose levels; NE, the agent did not induce a statistically significant decrease in incidence or multiplicity, or increase in latency at any dose level tested. See the text of this paper for detailed descriptions of the evaluation criteria for each model.

[4] The number in [ ] refers to additional publications describing the test result.

[5] The letter in ( ) describes the treatment schedule of the test agent relative to that of the carcinogen: A, the agent was administered from one week pre-carcinogen to one week post-carcinogen; B, the agent was administered from one week post-carcinogen to the end of the study. All other test results were obtained on treatment schedule C, in which the agent was administered from one week pre-carcinogen to the end of the study (*i.e.*, throughout the whole test period).

results were obtained in testing by schedules A and B. These results are annotated in Table I.

The agents tested may be classified into various pharmacological and chemical structural categories. These categories include antiestrogens, antiinflammatories (*e.g.*, NSAIDs), antioxidants, arachidonic acid metabolism inhibitors, GST and GSH enhancers, ODC inhibitors, protein kinase C inhibitors, retinoids and carotenoids, organosulfur compounds, calcium compounds, vitamin D_3 and analogs, and phenolic compounds (*e.g.*, flavonoids). For each model, the performance of classes in which at least five agents produced conclusive test results are reviewed in conjunction with the models. In the analysis of the activities by class, emphasis is placed on positive results, since lack of effect may be attributed to factors other than the intrinsic activity of the agent (*e.g.*, poor absorption, inadequate dose).

Hamster Respiratory Tract

DEN-Induced Tracheobronchial Tumors Twelve agents were tested in this model; four were considered positive and eight were ineffective in inhibiting DEN-induced lung tumorigenesis. All agents were tested according to schedule C. Agents with chemopreventive activity were bismuthiol I, 4-HPR [15,34], oltipraz [34], and sodium selenite. Based on the limited available test results, no specific class of agents can be considered to be particularly effective in this assay. However, the positive results suggest additional testing that might be done to evaluate the mechanisms of chemopreventive activity in this model.

Oltipraz, a synthetic dithiolthione, has previously been shown to inhibit DEN-induced lung tumorigenesis [35]. In Chemoprevention Branch testing, this agent was active in all the models discussed here (see Table I), and it produced 100% tumor inhibition in the DEN-lung model at 600 mg/kg diet after 25 weeks of treatment [34]. Although the mechanism of action of oltipraz in lung is not fully understood, its activity may be attributable to its enhancement of GSH and GST and other phase II metabolic enzymes [36–40]. Oltipraz was also active in the MNU-trachea model, as were other GSH-enhancing agents including diallyl disulfide and NAC. Further testing of such agents in lung may be warranted.

The retinoid 4-HPR was active at 1 mmol/kg diet in this model [15]. It was ineffective at lower doses of 0.25 and 0.5 mmol/kg diet when administered for 25 weeks [34]. The role of retinoids in prevention of aerodigestive tract cancers and the potential involvement of nuclear retinoic acid receptors have recently been reviewed [41,42]. Inhibition of squamous cell differentiation by retinoic acid is believed to be mediated through specific retinoid receptors in both epithelial and tracheobronchial cells. Jetten and colleagues [43] have studied the role of retinoids in control of squamous tissue differentiation in tracheobronchial and epidermal epithelial cells. Using explant or cell culture systems, these investigators demonstrated that retinoic acid suppresses squamous cell markers (*e.g.*, involucrin, transglutaminase type I, cholesterol sulfate, and keratin 13) at nanomolar concentrations.

Bismuthiol I [44], oltipraz [39], and sodium selenite [45] are antioxidants, providing a rationale for evaluating other antioxidants in the DEN model. Particularly, the chemopreventive activity of sodium selenite may be related to its antioxidant potential via its role in the active site of GSH peroxidase [45]. Besides its chemopreventive activity in other tissues [1,2], selenite was previously found effective against BOP-induced adenocarcinomas in rat lung [46].

MNU-Induced Tracheobronchial Tumors

Twenty agents were tested in this model; five significantly inhibited MNU-induced tracheobronchial tumors and 15 were ineffective. Positive agents were NAC, diallyl disulfide, ethylvanillin, fumaric acid, and oltipraz; all the positive results were obtained according to schedule C except for NAC, which was positive according to schedule B. Although no specific category of agents was most active in this assay, those categorized as GSH/GST enhancers—NAC, diallyl disulfide, oltipraz—appear to be most effective. As in the DEN-lung model, antioxidants may also prove effective. Generally, a positive result in this assay is highly regarded, since a massive carcinogen dose directly applied to the target tissue must be overcome to observe an effect.

In published studies NAC has been shown to inhibit urethane-induced lung tumors [47] and DMH-induced colon and small intestine tumors [48]. In Chemoprevention Branch-sponsored studies, it has also been active in rat mammary glands, rat colon and mouse bladder (see Table I). Like oltipraz, NAC [49] stimulates intracellular production of GSH and activity of GST; NAC is easily deacetylated to yield cysteine *in vivo*, which enhances GSH synthesis. These activities may be the basis of

its chemopreventive potential. The primary mechanism of action of diallyl disulfide appears to be via enhancement of GST activity [50,51]. Published studies have shown that diallyl disulfide was effective in preventing DEN-induced lung adenomas and forestomach papillomas and carcinomas in A/J mice [52]. In addition, it was shown to suppress DMH-induced colon cancer in mice [53], B(*a*)P-induced lung tumors in mice [54], and esophageal cancer in rats [55].

Several compounds with antioxidant activity—NAC [56], oltipraz, ethyl vanillin, and particularly, fumaric acid [57–59]—were active in the MNU-trachea model. Fumaric acid has been shown to have chemopreventive activity in mouse forestomach and lung [57] and rat liver [58,59]. The mechanism of action of fumaric acid is not fully understood, but is likely to be related to its antioxidant potential. It also appears to be effective in later stages of tumorigenesis, since in studies cited above it was administered after carcinogen treatment was completed.

Colon Studies: Aberrant Crypts Assay, Mouse and Rat Models

Aberrant Crypt Foci A total of 101 agents have been tested in the aberrant crypt focus assay. Most of the compounds were tested on treatment schedule A; a few were tested only on schedule B or both schedules A and B. In Table I, results are cited for each schedule by which the agent was tested. Fifty-seven agents were positive and 44 were negative. The categories of compounds producing high percentages of positive results are antiinflammatories (20/32, 62%), and particularly, NSAIDs (6/8, 75%), GSH/GST enhancers (11/19, 58%), ODC inhibitors (25/43, 58%), retinoids (6/6, 100%), calcium compounds (4/5, 80%), and vitamin D derivatives (3/5, 60%). Some of the results in the aberrant crypt foci assay have been published previously [20,21,60]. A major interest in the crypt foci assay is its use as a short-term model; therefore, it is important to determine its predictive value for results in some of the longer term models. Its predictive value for animal colon cancers is described in the following paragraphs on these models.

Rat Colon In the rat colon model, 18 agents were tested; 14 were positive and four were negative. All positive agents were active when tested by schedule C; DFMO and piroxicam were also tested by and active on schedule B. Among the various categories of agents tested, high percentages of positive results were obtained with antiinflammatories (7/9, 78%) and, particularly, NSAIDs (5/6, 82%), arachidonic acid metabolism inhibitors (8/9, 89%), antioxidants (10/12, 83%), GSH/GST enhancers (5/7, 71%), and ODC inhibitors (8/9, 88%).

Eight agents were positive in both the aberrant crypt foci and rat colon models. These agents were aspirin [21,61], DFMO [21,62,63], ibuprofen [21,64], ketoprofen [21,64], oltipraz [21,65,66], piroxicam [21,62–64,67,68], sulindac, and taurine [69]. Seven agents were not effective in the aberrant crypt foci assay, but were positive in the rat colon model: NAC [69], anethole trithione [69], ascorbyl palmitate, carbenoxolone, DHEA analog 8354 [63], diallyl disulfide [69], and ellagic acid [63]. Only one agent, dimethyl fumarate, was positive in the aberrant crypt foci assay but negative in the rat colon model. One agent, 4-methoxyphenol, was negative in both studies. Based on these results, the aberrant crypt foci assay was accurate in predicting results of the rat colon assay in 9/17 cases, or 53% of the time. However, the correlation may prove to be much higher for specific classes of compounds. For example, all five NSAIDs tested were positive in both models.

Mouse Colon Thirty-six agents have been tested in the mouse colon model; 11 were positive and 25 were negative. Except for fluocinolone acetonide (schedule B), 18β-glycyrrhetinic acid (schedules A and B), and oltipraz (schedule A), the positive results were obtained according to schedule C. Agents categorized as antiinflammatory (5/7, 70%; interestingly, in contrast to the other colon models, indomethacin—the one NSAID tested in this model—was negative) and arachidonic acid metabolism inhibitors (7/12, 58%) produced relatively high percentages of positive test results in this model.

Thirty-four agents were tested in both the aberrant crypt foci assay and the mouse colon model. Nine were positive and 15 were negative in both assays (24/34, 71% accuracy). Overall, of 38 agents tested in the aberrant crypts assay and either the rat or mouse colon model, 10 were positive in both and 14 were negative in both (24/38, 63% accuracy).

NSAIDs were one of the most active classes of agents in the colon assays. Besides the results reported here, there have been other studies cited in the literature in which NSAIDs have shown chemopreventive activity in colon and small intestines [70–73]. They also inhibit the induction of tumors in rat [74,75] and mouse (see below) urinary blad-

der, hamster buccal pouch [76,77], rat mammary glands [78–81], mouse skin [82–85], mouse duodenum [86], and hamster esophagus [87], pancreas [88], and uterine cervix [89].

NSAIDs are predominantly inhibitors of prostaglandin biosynthesis. 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.*, 90–93]. The mechanism(s) may involve not only reductions in growth-promoting tissue prostaglandin levels, but also relief of suppressed immune surveillance [94,95] and inhibition of oxidation (activation) of proximate carcinogens [93, 96,97].

ODC inhibitors were highly active in both the aberrant crypts assay and the rat colon model. DFMO, a specific irreversible inhibitor of ODC [*e.g.*, 98,99], has been shown to inhibit AOM-induced colon cancer in rats, both in studies reported here [62,63] and those cited elsewhere [72,99]. Additionally, DFMO has been shown to be chemopreventive in the mouse colon [100,101], mouse skin [102], rat [100,103,104] and mouse urinary bladder [see below and 32,33], and mouse [105,106] and rat mammary glands [see below and 100] models.

Calcium compounds were active in the aberrant crypts assay. Increased dietary calcium has been shown to suppress the hyperproliferation of colonic epithelium induced by bile salts and fatty acids [*e.g.*, 107–110]. Calcium lactate at 3.2 g/kg diet has been shown to inhibit AOM-induced colon adenocarcinomas in Fischer rats [111]. One proposed mechanism is the binding of calcium to fatty acids, thus preventing promotion of carcinogenesis by fatty acid damage to the epithelium [112,113].

All the retinoids tested (4-HPR, 9-*cis*-retinoic acid, 13-*cis*-retinoic acid, all-*trans*-retinoic acid, Ro 16-9100, vitamin A) were positive in the aberrant crypts assay, but these agents have not been well-tested in the colon. They demonstrate chemopreventive activity in mammary glands, respiratory tract, and urinary bladder. Activity in the colon aberrant crypts assay may prove useful in predicting the activity of retinoids in these other tissues.

DMBA- and MNU-Induced Mammary Tumors in Rats

DMBA-Induced Tumors Fourteen agents were tested in the DMBA model. Seven (50%) were positive; all were tested according to schedule C. Positive agents were anethole trithione, (+)-catechin, DFMO [100], 4-HPR, indole-3-carbinol, oltipraz, and sodium selenite.

MNU-Induced Tumors In the MNU model, 92 agents were tested; 30 were positive and 62 were negative. The positive agents were NAC, aminogluthetimide, BASF-47851, 2-bromo-α-ergocryptine, carbenoxolone, β-carotene (injectable), curcumin, DFMO, DHEA [114], DHEA analog 8354 [114], ethylvanillin, fish oils, fumaric acid, calcium glucarate, 18β-glycyrrhetinic acid, 4-HPR [24,115,116], ibuprofen, indole-3-carbinol, sodium molybdate, oltipraz, praziquantel, progesterone [117], Ro 16-9100, Ro 19-2968, RU 16117 [117], tamoxifen (and tamoxifen citrate) [24,115,117], temaroten, toremifene [117], and (+)-vorozole [118]. Except for β-carotene (injectable, schedule B), all positive results were obtained according to schedule C.

Antiproliferation is widely believed to be an important chemopreventive mechanism in breast cancer. Antiproliferative agents such as DFMO, retinoids (4/7, 66% positive), and antiestrogens (8/13, 62% positive) showed significant chemopreventive activity against mammary carcinogenesis in tests carried out in the Chemoprevention Branch program. These classes of agents have also demonstrated activity against mammary gland carcinogenesis in other published studies [reviewed in 5].

DFMO was active in both the DMBA and MNU models; its chemopreventive activity is described throughout this paper. The retinoid 4-HPR was also active in both the DMBA and MNU models; other retinoids—Ro 16-9100, Ro 19-2968, BASF-47851, and temaroten—were active in the MNU model. Further, the carotenoid β-carotene administered subcutaneously at 10 mg/kg-bw, was active in the MNU model; it may derive its activity in part from conversion to vitamin A [119,120]. Other published studies have shown that β-carotene reduces mammary gland carcinogenesis in the DMBA-induced rat [121,122] and virus-associated mouse mammary models [123]. Another carotenoid without provitamin A activity, canthaxanthine, inhibited mammary adenocarcinomas in the DMBA model [124].

Several mechanisms of antiestrogenic activity are represented among the agents positive in the rat mammary carcinogenesis models. Tamoxifen [125,126], toremifene (the chlorinated analog of tamoxifen) [127], and RU 16117 [128] are direct antagonists at the estrogen receptor. All these agents were active in the MNU model, and all have previously demonstrated activity in rat mammary glands models in other studies reported in the literature (*e.g.*, tamoxifen [129–131], toremifene

[132], RU 16117 [133,134]). Of the five receptor antagonists tested, four (80%) were active. Progesterone, which was positive in the MNU model, represents the class of antiestrogens with estrogen-opposing activity. This agent also has been previously found to inhibit MNU-induced rat mammary tumors [135–137].

Another antiestrogen mechanism is inhibition of the enzyme steroid aromatase. Aromatase, a cytochrome P-450-dependent enzyme, catalyzes the first step in estrogen biosynthesis in humans, namely the C-19 hydroxylation and subsequent oxidative cleavage and aromatization of the androgens androstenedione and testosterone to estrone and estradiol, respectively [138,139]. Two of the six aromatase inhibitors tested were positive in the rat mammary models. These results may underestimate the potency of aromatase inhibitors in preventing mammary cancers, since only one of the negative compounds (4-hydroxyandrost-4-ene-3,17-dione) was specifically an aromatase inhibitor, and the other three were polyphenols (quercetin, rutin, NDGA) that were possibly not absorbed and were not bioavailable to inhibit the enzyme in relevant tissues under the conditions of the test. Aminoglutethimide and (+)-vorozole were the positive aromatase inhibitors. (+)-Vorozole is particularly interesting, since it was highly active (90% reduction in tumor incidence, 98% reduction in tumor multiplicity) at low doses (2.5 and 5.0 mg/kg-bw/day) compared with its MTD [116]. The mixed isomer form of vorozole (R 76713) has previously been reported to have chemopreventive efficacy against MNU- and DMBA-induced mammary tumors in rats [140,141].

A fourth mechanism of antiestrogenic activity is the stimulation of steroid hydroxylation and excretion, particularly the induction of enzymes responsible for 2-hydroxylation of estradiol, leading to increased excretion of estradiol metabolites. Indole-3-carbinol, active in both the DMBA and MNU models, promotes steroid 2-hydroxylation [142,143]. Indole-3-carbinol has previously been reported to be chemopreventive in the DMBA-induced rat mammary cancer model [144].

DHEA, DHEA analog 8354 and (+)-catechin are interesting examples of agents active in rat mammary models. DHEA and DHEA analog 8354 were very active in the MNU model; they also were active in mouse and rat colon, respectively. Schwartz and his colleagues [145–150,155], as well as other investigators [151–154], have demonstrated the chemopreventive activity of the androgen DHEA in numerous animal models. DHEA is a potent inhibitor of G6PDH, which catalyzes formation of extramitochondrial NAD(P)H and ribose 5-phosphate. Schwartz [155] has hypothesized two ways in which inhibition of G6PDH may mediate the chemopreventive activity of DHEA. First, DHEA inhibits the activity of carcinogens such as B(*a*)P, AFB_1, and DMBA which require metabolic activation via mixed-function oxidases [155–158]. Mixed-function oxidases require NAD(P)H as a cofactor. Since inhibition of G6PDH reduces the formation of NAD(P)H, it consequently reduces the activity of mixed-function oxidases and the activation of certain carcinogens. Secondly, DHEA also inhibits tumor promotion and proliferative activity induced by TPA [149,159]. Cell proliferation requires NAD(P)H-dependent DNA synthesis, and DNA synthesis in mouse epidermis and mammary tissue is also inhibited by DHEA [160,161]. Accordingly, reduction of the NAD(P)H pool by inhibition of G6PDH could inhibit carcinogen-induced cell proliferation. Unfortunately, the chemopreventive potential of DHEA may be compromised by some undesirable pharmacological effects—potent hormonal [162], liver-enlarging [163], and peroxisome-proliferating activities [164]. To eliminate these side effects while preserving chemopreventive activity, Schwartz designed several analogs [155,161,163]; DHEA analog 8354 (fluasterone) appears to be particularly promising as it does not have the same degree of androgenic and liver toxicity as DHEA [163]. The analog is a more potent inhibitor of tumor initiation and promotion in the DMBA/TPA mouse skin model than DHEA [159].

Based on positive results in *in vitro* screens for chemoprevention and those in animal efficacy tests cited in the published literature [165–174], the flavonoids would be assumed to have activity in the NCI *in vivo* efficacy models. Mechanistically, they have a wide range of activities including antioxidant potential implicated in inhibition of carcinogen activation and scavenging of electrophiles. However, thus far, positive results have been obtained only in colon (quercetin, rutin, and the isoflavone, genistein—see Table I) and in the DMBA-induced rat mammary gland with (+)-catechin. These results are representative of some of the precautions that should be taken in designing and interpreting animal screening results for chemopreventives. That is, one possible explanation for lack of activity is the metabolism of the flavonoids *in vivo*. They are generally not well absorbed and tend to undergo enterohepatic circulation. Thus, given orally, they are most likely to be active in tissues to which they have access such as the colon,

and against carcinogens which are activated in the liver, such as DMBA. This interpretation fits well with the other published results cited above indicating that flavonoids are positive in skin on topical application and against liver-activated carcinogens.

DMBA-Induced Skin Tumor Model

Only thirteen agents have been tested in the DMBA-skin model; six were positive and seven were considered to be negative. Positive agents were benzyl isothiocyanate, 18β-glycyrrhetinic acid, 4-HPR, oltipraz, piroxicam, and propyl gallate . All positive results were obtained according to schedule C, except for propyl gallate (schedule B). Too few compounds have been tested to define chemical classes with chemopreventive activity in this model, and except for benzyl isothiocyanate, all the positive compounds have marked antiproliferative activity. Skin chemopreventive activity of 4-HPR [28,175] and 18β-glycyrrhetinic acid [176–178] is well documented, although such activity has not been reported previously for the other positive compounds. For example, several NSAIDs have been found to have chemopreventive activity in mouse skin cancer models [*e.g.*, 82–85]; however, the chemopreventive activity of piroxicam in this model has not been published in the literature. Likewise, the activities of benzyl isothiocyanate, propyl gallate, and oltipraz have not been reported previously.

OH-BBN-Induced Bladder Tumor Model

Forty-four agents were tested in this mouse model; 11 were positive and 33 were negative. Positive agents were NAC, bismuthiol I, DFMO [32, 33,100,179], ibuprofen, indomethacin [180], ketoprofen, 2-mercaptoethanesulfonate, sodium molybdate [179], oltipraz [33,179], piroxicam [32,179], and sulindac. All the agents with chemopreventive activity were tested according to schedule C. Two groups of agents showed significant activity in bladder and may be worthy of further evaluation in this target. The first is NSAIDS; of the six tested, five were positive (83%)—ibuprofen, indomethacin, ketoprofen, piroxicam, and sulindac. Further, aspirin, which was negative in the NCI study, had previously shown activity in the OH-BBN-induced bladder model in rats [181]. Agents containing a thiol moiety were also active in the bladder model. Just as for NSAIDs, four of five thiols tested (80%), were positive—NAC, bismuthiol I, 2-mercaptoethanesulfonate, and oltipraz.

DISCUSSION AND FUTURE DIRECTIONS

The primary goal of the Chemoprevention Branch drug development program is the development of safe and effective chemopreventive agents for human use. This effort has been carried out in several steps beginning with agent selection and prioritization to progression into the clinical trials. One of the steps in this path involves testing of the potential agents as chemopreventives in a battery of *in vivo* screens and models. There has been continuous progress in developing and designing shorter, more precise, and quality-controlled animal assays to examine the efficacy of chemopreventive agents so that drug development can proceed more efficiently. To date, as described in this paper, the primary focus has been on such targets as lung, colon, mammary glands, skin, and bladder; currently, other models including prostate and pancreas are being evaluated. In the next few years, other targets of high relevance to human cancers, in particular, brain, blood (leukemia), lymphatic system (non-Hodgkin's lymphoma), and skin (melanoma), will be addressed.

Several factors are considered in designing the *in vivo* models and screens. These include conducting experiments in relatively short-term (less than six months) studies, using different target organs, selecting target tissues in animals with high relevance to human cancers, and using long-term, low doses of carcinogen to model human exposure. In selecting and prioritizing agents for testing, other parameters such as procurement, safety, and pharmacokinetics are also considered. Perhaps more emphasis should be placed on understanding the pharmacokinetics, structure-activity relationships, and mechanism of action at the biochemical level in order to elucidate organ specificity and assist in development of novel chemopreventive agents. The importance of pharmacokinetics and metabolism was illustrated above in the discussion of test results obtained with flavonoids. The design of the experiments may also be modified so that the agent can be administered by different routes of administration (*e.g.*, intragastric instead of dietary) or may be pair-fed allowing evaluation at higher doses.

Another important aspect of screening in animal models is evaluating the potential cancer target specificity of various pharmacological and chemical structural categories of agents. The results of such

evaluation provide leads to new drugs, as well as suggesting the most appropriate clinical development path for promising agents. The testing described in this paper has helped characterize the chemopreventive spectra of several categories of drugs. For example, GSH-enhancing agents and antioxidants appear to have high potential for chemoprevention in lung. NSAIDs and other inflammatory agents are promising in colon, and NSAIDs are highly efficacious in bladder. Also, retinoids and antiestrogens (as would be expected) are efficacious in mammary gland.

In addition to single agents, combinations of agents have been utilized to measure chemopreventive efficacy; these combinations offer the possibility of additive or synergistic efficacy with less toxicity. Combinations are also attractive since they offer the possibility of combining agents with different primary mechanisms of action, such as antiestrogens and antiproliferatives, for broad spectrum preventive effects.

REFERENCES

1. Bagheri, D., Doeltz, M.K., Fay, J.R., Helmes, C.T., Monasmith, L.A., and Sigman, C.C. Database of inhibitors of carcinogenesis. *J. Environ. Sci. Health* C6 (3), 1989.
2. Kelloff, G.J., Boone, C.W., Steele, V.E., Fay, J.R., and Sigman, C.C. Inhibition of chemical carcinogenesis. In: Arcos, J., Argus, M., and Woo, Y. (eds.), *Chemical Induction of Cancer: Modulation and Combination Effects*, Boston, MA: Birkhäuser Boston, Inc., 1994, *in press*.
3. Kelloff, G.J., Boone, C.W., Malone, W.F., and Steele, V. Recent results in preclinical and clinical drug development of chemopreventive agents at the National Cancer Institute. In: Wattenberg, L., Lipkin, M., Boone, C.W., and Kelloff, G.J. (eds.), *Cancer Chemoprevention*, Boca Raton, FL: CRC Press, 1992, pp. 41–56.
4. Kelloff, G.J., Boone, C.W., Crowell, J.A., Steele, V.E., Lubet, R., and Sigman, C.C. Chemopreventive drug development: Perspectives and progress. *Cancer Epidemiol. Biomarkers Prev.* 3: 85–98, 1994.
5. Kelloff, G.J., Boone, C.W., Steele, V.E., Crowell, J.A., Lubet, R., and Sigman, C.C. Progress in cancer chemoprevention: Perspectives on agent selection and short-term clinical intervention trials. *Cancer Res.* 54: 2015s–2024s, 1994.
6. Kelloff, G.J., Boone, C.W., Steele, V.E., Fay, J.R., Lubet, R.A., Crowell, J.A., and Sigman, C.C. Mechanistic considerations in cancer chemopreventive drug development. *J. Cell Biochem., in press*.
7. Kelloff, G.J., Malone, W.F., Boone, C.W., Sigman, C.C., and Fay, J.R. Progress in applied chemoprevention research. *Semin. Oncol.* 17: 438–455, 1990.
8. Boone, C.W., Kelloff, G.J., and Malone, W.F. Identification of candidate cancer chemopreventive agents and their evaluation in animal models and human clinical trials: A review. *Cancer Res.* 50: 2–9, 1990.
9. Boone, C.W., Steele, V.E., and Kelloff, G.J. Screening for chemopreventive (anticarcinogenic) compounds in rodents. *Mutat. Res.* 267: 251–255, 1992.
10. Kelloff, G.J., Boone, C.W., Malone, W.F., and Steele, V. Recent results in preclinical and clinical drug development of chemopreventive agents at the National Cancer Institute. *Basic Life Sci.* 61: 373–386, 1993.
11. Freedman, L.S., Midthune, D.S., Brawn, C.C., Steele, V., and Kelloff, G.J. Statistical analysis of animal cancer chemopreventive experiments. *Biometrics* 49: 259–268, 1993.
12. Chevalier, S., Tuchweber, B., Bhat, P.V., and Lacroix, A., Dietary restriction reduces the incidence of MNU-induced mammary tumors and alters retinoid tissue concentrations in rats. *Nutr. Cancer* 20: 187–196, 1993.
13. Klurfeld, D.M., Welch, C.B., Lloyd, L.M., and Kritchevsky, D. Inhibition of DMBA-induced mammary tumorigenesis by caloric restriction in rats fed high-fat diets. *Int. J. Cancer* 43: 922–925, 1993.
14. Moon, R.C. and Mehta, R.G. Chemoprevention of experimental carcinogenesis in animals. *Prev. Med.* 18: 576–591, 1989.
15. Moon, R.C., Rao, K.V.N, Detrisac, C.J., and Kelloff, G.J. Hamster lung cancer model of carcinogenesis and chemoprevention. *Adv. Exp. Med. Biol.* 320: 55-61, 1992.
16. Schüller, H.M. and McMahon, J.B. Inhibition of *N*-nitrosodiethylamine-induced respiratory tract carcinogenesis by piperonylbutoxide in hamsters. *Cancer Res.* 45: 2807–2812, 1985.
17. Reddy, B.S. and Maeura, Y. Tumor promotion by dietary fat in azoxymethane-induced colon cancer. Carcinogenesis in female F-344 rats: Influence of amount and source of dietary fat. *J. Natl. Cancer Inst.* 72: 745–750, 1984.
18. Reddy, B.S. and Maeura, Y. Dose-response studies of the effect of dietary butylated hydroxyanisole on colon carcinogenesis induced by methylazoxymethanol acetate in female CF1 mice. *J. Natl. Cancer Inst.* 72: 1181–1187, 1984.
19. Bird, R.P. Observation and quantification of aberrant crypts in the murine colon treated with a colon carcinogen: Preliminary findings. *Cancer Lett.* 37: 147–151, 1987.
20. Pereira, M.A., Barnes, L.H., Rassman, V.L., Kelloff, G.J., and Steele, V.E. Use of azoxymethane-induced foci of aberrant crypts in rat colon to identify potential cancer chemopreventive agents. *Carcinogenesis* 15: 1049–1054, 1994.
21. Wargovich, M.J., Harris, C., Chen, C.-D., Palmer, C., Steele, V.E., and Kelloff, G.J. Growth kinetics and chemoprevention of aberrant crypts in the rat colon. *J Cell. Biochem.* 16 (Suppl. G): 51–56, 1994.
22. Gullino, P.M., Pettigrew, H.M., and Grantham, F.H. *N*-Nitrosomethylurea as mammary gland carcinogen in rats. *J. Natl. Cancer Inst.* 54: 401–414, 1975.
23. McCormick, D.L., Adamowski, C.B., Fiks, A., and

Moon, R.C. Lifetime dose-response relationships for mammary tumor induction by a single administration of *N*-methyl-*N*-nitrosourea. *Cancer Res.* 41: 1690–1694, 1981.

24. Zarbl, H., Sukumar, S., Arthur, A.V., Martin-Zanca, D., and Barbacid, M. Direct mutagenesis of Ha-*ras*-1 oncogenes by *N*-nitroso-*N*-methylurea during initiation of mammary carcinogenesis in rats. *Nature* 315: 382–385, 1985.
25. Lu, S.-J. and Archer, M.C. *ras* Oncogene activation in mammary carcinomas induced by *N*-methyl-*N*-nitrosourea in Copenhagen rats. *Mol. Carcinog.* 6: 260–265, 1992.
26. Moon, R.C., Kelloff, G.J., Detrisac, C.J., Steele, V.E., Thomas, C.F., and Sigman, C.C. Chemoprevention of MNU-induced mammary tumors in the mature rat by 4-HPR and tamoxifen. *Anticancer Res.* 12: 1147–1153, 1992.
27. Warren, B.S. and Slaga, T.J. Mechanisms of inhibition of tumor progression. *Basic Life Sci.* 61: 279–289, 1993.
28. McCormick, D.L. and Moon, R.C. Antipromotional activity of dietary *N*-(4-hydroxyphenyl)retinamide in two-stage skin tumorigenesis in CD-1 and SENCAR mice. *Cancer Lett.* 31: 133–138, 1986.
29. Becci, P.J., Thompson, H.J., Strum, J.M., Brown, C.C., Sporn, M.B., and Moon, R.C. *N*-Butyl-*N*-(4-hydroxybutyl)nitrosamine-induced urinary bladder cancer in C57BL/6 x DBA/2 F_1 mice as a useful model for study of chemoprevention of cancer with retinoids. *Cancer Res.* 41: 927–932, 1981.
30. McCormick, D.L., Ronan, S.S., Becci, P.J., and Moon, R.C. Influence of total dose and dose schedule on induction of urinary bladder cancer in the mouse by *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine. *Carcinogenesis* 2: 251–254, 1981.
31. Moon, R.C., McCormick, D.L., Becci, P.J., Shealy, Y.F., Frickel, F., Paust, J., and Sporn, M.B. Influence of 15 retinoic acid amides on urinary bladder carcinogenesis in the mouse. *Carcinogenesis* 3: 1469–1472, 1982.
32. Moon, R.C., Kelloff, G.J., Detrisac, C.J., Steele, V.E., Thomas, C.F., and Sigman, C.C. Chemoprevention of OH-BBN-induced bladder cancer in mice by piroxicam. *Carcinogenesis* 14: 1487–1489, 1993.
33. Moon, R.C., Kelloff, G.J., Detrisac, C.J., Steele, V.E., Thomas, C.F., and Sigman, C.C. Chemoprevention of OH-BBN-induced bladder cancer in mice by oltipraz, alone and in combination with 4-HPR and DFMO. *Anticancer Res.* 14: 5–11, 1994.
34. Moon, R.C., Rao, K.V.N., Detrisac, C.J., Kelloff, G.J., Steele, V.E., and Doody, L.A. Chemoprevention of respiratory tract neoplasia in the hamster by oltipraz, alone and in combination. *Int. J. Oncol.* 4: 661–667, 1994.
35. Wattenberg, L.W. and Bueding, E. Inhibitory effects of 5-(2-pyrazinyl)-4-methyl-1,2-dithiol-3-thione (Oltipraz) on carcinogenesis induced by benzo[*a*]pyrene, diethylnitrosamine and uracil mustard. *Carcinogenesis* 7: 1379–1381, 1986.
36. Ansher, S.S., Dolan, P., and Bueding, E. Chemoprotective effects of two dithiolthiones and of butylhydroxyanisole against carbon tetrachloride and acetaminophen toxicity. *Hepatology* 3: 932–935, 1983.
37. Kensler, T.W., Egner, P.A., Trush, M.A., Bueding, E., and Groopman, J.D. Modification of aflatoxin B_1 binding to DNA *in vivo* in rats fed phenolic antioxidants, ethoxyquin and a dithiothione. *Carcinogenesis* 6: 759–763, 1985.
38. Kensler, T.W., Egner, P.A., Dolan, P.M., Groopman, J.D., and Roebuck, B.D. Mechanism of protection against aflatoxin tumorigenicity in rats fed 5-(2-pyrazinyl)-4-methyl-1,2-dithiol-3-thione (oltipraz) and related 1,2-dithiol-3-thiones and 1,2-dithiol-3-ones. *Cancer Res.* 47: 4271–4277, 1987.
39. Roebuck, B.D., Liu, Y.-L., Rogers, A.E., Groopman, J.D., and Kensler, T.W. Protection against aflatoxin B_1-induced hepatocarcinogenesis in F344 rats by 5-(2-pyrazinyl)-4-methyl-1,2-dithiole-3-thione (oltipraz): Predictive role for short-term molecular dosimetry. *Cancer Res.* 51: 5501–5506, 1991.
40. Liu, Y.L., Roebuck, B.D., Yager, J.D., Groopman, J.D., and Kensler, T.W. Protection by 5-(2-pyrazinyl)-4-methyl-1,2-dithiol-3-thione (Oltipraz) against the hepatotoxicity of aflatoxin B_1 in the rat. *Toxicol. Appl. Pharmacol.* 93: 442–451, 1988.
41. Lotan, R. Suppression of squamous cell carcinoma growth and differentiation by retinoids. *Cancer Res.* 54: 1987s–1990s, 1994.
42. Lippman, S.M., Benner, S.E., and Hong, W.K. Retinoid chemoprevention studies in upper aerodigestive tract and lung carcinogenesis. *Cancer Res.* 54: 2025s–2028s, 1994.
43. Jetten, A.M., Nervi, C., and Vollberg, T.M. Control of squamous differentiation in tracheobronchial and epidermal epithelial cells: Role of retinoids. *Monogr. Natl. Cancer Inst.* 13: 93–100, 1992.
44. Rauen, V.H.M., Schriewer, H., Tegtbauer, U., and Lasana, J.E. The effect of aliphatic and heterocyclic mercapto compounds on lipid peroxidation in liver injury of the rat by CCl_4. *Arzneimittelforschung* 23: 145–148, 1973.
45. Borek, C., Ong, A., Mason, H., Donahue, L., and Biaglow, J.E. Selenium and vitamin E inhibit radiogenic and chemically induced transformation *in vitro* via different mechanisms. *Proc. Natl. Acad. Sci. USA* 83: 1490–1494, 1986.
46. Birt, D.F., Lawson, T.A., Julius, A.D., Runice, C.E., and Salmasi, S. Inhibition by dietary selenium of colon cancer induced in the rat by bis(2-oxopropyl)-nitrosamine. *Cancer Res.* 42: 4455–4459, 1982.
47. De Flora, S., Astengo, M., Serra, D., and Bennicelli, C. Inhibition of urethan-induced lung tumors in mice by dietary *N*-acetylcysteine. *Cancer Lett.* 32: 235–241, 1986.
48. Wilpart, M., Speder, A., and Roberfroid, M. Antiinitiation activity of *N*-acetylcysteine in experimental colonic carcinogenesis. *Cancer Lett.* 31: 319–324, 1986.
49. De Flora, S., Rossi, G.A., and De Flora, A. Metabolic, desmutagenic and anticarcinogenic effects of *N*-acetylcysteine. *Respiration* 50: 43–49, 1986.

50. Huh, K., Lee, S.I., Park, J.M., and Kim, S.H. Effect of diallyl disulfide on the hepatic glutathione *S*-transferase activity in rat: Diallyl disulfide effect on the glutathione *S*-transferase. *Arch. Pharmacol. Res.* 9: 205–209, 1986.
51. Sumiyoshi, H. and Wargovich, M.J. Chemoprevention of 1,2-dimethylhydrazine-induced colon cancer in mice by naturally occurring organosulfur compounds. *Cancer Res.* 50: 5084–5087, 1990.
52. Wattenberg, L.W., Sparnins, V.L., and Barany, G. Inhibition of *N*-nitrosodiethylamine carcinogenesis in mice by naturally occurring organosulfur compounds and monoterpenes. *Cancer Res.* 49: 2689–2692, 1989.
53. Wargovich, M.J. Diallyl sulfide, a flavor component of garlic (*Allium sativum*), inhibits dimethylhydrazine-induced colon cancer. *Carcinogenesis* 8: 487–489, 1987.
54. Sparnins, V.L., Barany, G., and Wattenberg, L.W. Effects of organosulfur compounds from garlic and onions on benzo[*a*]pyrene-induced neoplasia and glutathione *S*-transferase activity in the mouse. *Carcinogenesis* 9: 131–134, 1988.
55. Tudek, B., Bird, R.P., and Bruce, W.R. Foci of aberrant crypts in the colons of mice and rats exposed to carcinogens associated with foods. *Cancer Res.* 49: 1236–1240, 1989.
56. Aruoma, O.I., Halliwell, B., Hoey, B.M., and Butler, J. The antioxidant action of *N*-acetylcysteine: Its reaction with hydrogen peroxide, hydroxyl radical, superoxide, and hypochlorous acid. *Free Radic. Biol. Med.* 6: 593–597, 1989.
57. Kuroda, K., Kanisawa, M., and Akao, M. Inhibitory effect of fumaric acid on forestomach and lung carcinogenesis by a 5-nitrofuran naphthyridine derivative in mice. *J. Natl. Cancer Inst.* 69: 1317–1320, 1982.
58. Kuroda, K., Terao, K., and Akao, M. Inhibitory effect of fumaric acid on 3-methyl-4'-(dimethylamino)-azobenzene-induced hepatocarcinogenesis in rats. *J. Natl. Cancer Inst.* 71: 855–857, 1983.
59. Kuroda, K., Terao, K., and Akao, M. Inhibitory effect of fumaric acid on hepatocarcinogenesis by thioacetamide in rats. *J. Natl. Cancer Inst.* 79: 1047–1051, 1987.
60. Pereira, M.A. and Khoury, M.D. Prevention by chemopreventive agents of azoxymethane-induced foci of aberrant crypts in rat colon. *Cancer Lett.* 61: 27–33, 1991.
61. Reddy, B.S., Rao, C.V., Rivenson, A., and Kelloff, G. Inhibitory effect of aspirin on azoxymethane-induced colon carcinogenesis in F344 rats. *Carcinogenesis* 14: 1493–1497, 1993.
62. Reddy, B.S., Nayini, J., Tokumo, K., Rigotty, J., Zang, E., and Kelloff, G. Chemoprevention of colon carcinogenesis by concurrent administration of piroxicam, a nonsteroidal antiinflammatory drug with D,L-α-difluoromethylornithine, an ornithine decarboxylase inhibitor, in diet. *Cancer Res.* 50: 2562–2568, 1990.
63. Rao, C.V., Tokumo, K., Rigotty, J., Zang, E., Kelloff, G., and Reddy, B.S. Chemoprevention of colon carcinogenesis by dietary administration of piroxicam, α-difluoromethylornithine, 16α-fluoro-5-androsten-17-one, and ellagic acid individually and in combination. *Cancer Res.* 51: 4528–4534, 1991.
64. Reddy, B.S., Tokumo, K., Kulkarni, N., Aligia, C., and Kelloff, G. Inhibition of colon carcinogenesis by prostaglandin synthesis inhibitors and related compounds. *Carcinogenesis* 13: 1019–1023, 1992.
65. Rao, C.V., Tokomo, K., Kelloff, G., and Reddy, B.S. Inhibition by dietary oltipraz of experimental intestinal carcinogenesis induced by azoxymethane in male F344 rats. *Carcinogenesis* 12: 1051–1055, 1991.
66. Rao, C.V., Rivenson, A., Katiwalla, M., Kelloff, G.J., and Reddy, B.S. Chemopreventive effect of oltipraz during different stages of experimental colon carcinogenesis induced by azoxymethane in male F344 rats. *Cancer Res.* 53: 2502–2506, 1993.
67. Singh, J., Kelloff, G., and Reddy, B.S. Intermediate biomarkers of colon cancer: Modulation of expression of *ras* oncogene by chemopreventive agents during azoxymethane-induced colon carcinogenesis. *Carcinogenesis* 14: 699–704, 1993.
68. Reddy, B.S., Maruyama, H., and Kelloff, G. Dose-related inhibition of colon carcinogenesis by dietary piroxicam, a nonsteroidal antiinflammatory drug, during different stages of rat colon tumor development. *Cancer Res.* 47: 5340–5346, 1987.
69. Reddy, B.S., Rao, C.V., Rivenson, A., and Kelloff, G.J. Chemoprevention of colon carcinogenesis by organosulfur compounds. *Cancer Res.* 53: 3493–3498, 1993.
70. Narisawa, T., Sato, M., Tani, M., Kudo, T., Takahashi, T., and Goto, A. Inhibition of development of methylnitrosourea-induced rat colon tumors by indomethacin treatment. *Cancer Res.* 41: 1954–1957, 1981.
71. Pollard, M., Luckert, P.H., and Schmidt, M.A. The suppressive effect of piroxicam on autochthonous intestinal tumors in the rat. *Cancer Lett.* 21: 57–61, 1983.
72. Nigro, N.D., Bull, A.W., and Boyd, M.E. Inhibition of intestinal carcinogenesis in rats: Effect of difluoromethylornithine with piroxicam or fish oil. *J. Natl. Cancer Inst.* 77: 1309–1313, 1986.
73. Moorghen, M., Ince, P., Finney, K.J., Sunter, J.P., Appleton, D.R., and Watson, A.J. A protective effect of sulindac against chemically-induced primary colonic tumours in mice. *J. Pathol.* 156: 341–347, 1988.
74. Murasaki, G., Zenser, T.V., Davis, B.B., and Cohen, S.M. Inhibition by aspirin of *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide-induced bladder carcinogenesis and enhancement of forestomach carcinogenesis. *Carcinogenesis* 5: 53–55, 1984.
75. Sakata, T., Hasegawa, R., Johansson, S.L., Zenser, T.V., and Cohen, S.M. Inhibition by aspirin of *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide initiation and sodium saccharin promotion of urinary bladder carcinogenesis in male F344 rats. *Cancer Res.* 46: 3903–3906, 1986.
76. Perkins, T.M. and Shklar, G. Delay in hamster buccal pouch carcinogenesis by aspirin and indomethacin. *Oral Surg. Oral Med. Oral Pathol.* 53: 170–178, 1982.
77. Cornwall, H., Odukoya, O., and Shklar, G. Oral mu-

cosal tumor inhibition by ibuprofen. *J. Oral Maxillofac. Surg.* 41: 795–800, 1983.
78. Carter, C.A., Milholland, R.J., Shea, W., and Ip, M.M. Effect of the prostaglandin synthetase inhibitor indomethacin on 7,12-dimethylbenz(*a*)anthracene-induced mammary tumorigenesis in rats fed different levels of fat. *Cancer Res.* 43: 3559–3562, 1983.
79. McCormick, D.L., Madigan, M.J., and Moon, R.C. Modulation of rat mammary carcinogenesis by indomethacin. *Cancer Res.* 45: 1803–1808, 1985.
80. McCormick, D.L. and Wilson, A.M. Combination chemoprevention of rat mammary carcinogenesis by indomethacin and butylated hydroxytoluene. *Cancer Res.* 46: 3907–3911, 1986.
81. Noguchi, M., Taniya, T., Koyasaki, N., Kumaki, T., Miyazaki, I., and Mizukami, Y. Effects of the prostaglandin synthetase inhibitor indomethacin on tumorigenesis, tumor proliferation, cell kinetics, and receptor contents of 7,12-dimethylbenz(*a*)anthracene-induced mammary carcinoma in Sprague-Dawley rats fed a high- or low-fat diet. *Cancer Res.* 51: 2683–2689, 1991.
82. Viaje, A., Slaga, T.J., Wigler, M., and Weinstein, I.B. Effects of antiinflammatory agents on mouse skin tumor promotion, epidermal DNA synthesis, phorbol ester-induced cellular proliferation, and production of plasminogen activator. *Cancer Res.* 37: 1530–1536, 1977.
83. Fischer, S.M., Furstenberger, G., Marks, F., and Slaga, T.J. Events associated with mouse skin tumor promotion with respect to arachidonic acid metabolism: A comparison between SENCAR and NMRI mice. *Cancer Res.* 47: 3174–3179, 1987.
84. Furstenberger, G., Gross, M., and Marks, F. Eicosanoids and multistage carcinogenesis in NMRI mouse skin: Role of prostaglandins E and F in conversion (first stage of tumor promotion) and promotion (second stage of tumor promotion). *Carcinogenesis* 10: 91–96, 1989.
85. Lowe, N.J., Connor, M.J., Breeding, J., and Chalet, M. Inhibition of ultraviolet-B epidermal ornithine decarboxylase-induction and skin carcinogenesis in hairless mice by topical indomethacin and triamcinolone acetonide. *Cancer Res.* 42: 3941–3943, 1982.
86. Pollard, M. and Luckert, P.H. Effect of indomethacin on intestinal tumors induced in rats by the acetate derivative of dimethylnitrosamine. *Science* 214: 558–559, 1981.
87. Rubio, C.A. Antitumoral activity of indomethacin on experimental esophageal tumors. *J. Natl. Cancer Inst.* 72: 705–707, 1984.
88. Takahashi, M., Furukawa, F., Toyoda, K., Sato, H., Hasegawa, R., Imaida, K., and Hayashi, Y. Effects of various prostaglandin synthesis inhibitors on pancreatic carcinogenesis in hamsters after initiation with *N*-nitrosobis(2-oxopropyl)amine. *Carcinogenesis* 11: 393–395, 1990.
89. Ramesha Rao, A. and Hussain, S.P. Modulation of methylcholanthrene-induced carcinogenesis in the uterine cervix of mouse by indomethacin. *Cancer Lett.* 43: 15–19, 1988.
90. Jaffe, B.M. Prostaglandins and cancer: An update. *Prostaglandins* 6: 453–46, 1974.
91. Karmali, R.A. Review: Prostaglandins and cancer. *Prostaglandins Med.* 5: 11–28, 1991.
92. Thun, M.J., Namboodiri, M.M., and Heath, C.W., Jr. Aspirin use and reduced risk of fatal colon cancer. *N. Engl. J. Med.* 325: 1593–1596, 1991.
93. Zenser, T.V. and Davis, B.B. Arachidonic acid metabolism. *In:* Steele, V.E., Boone, C.W., Stoner, G.D., and Kelloff, G.J. (eds.), *Cellular and Molecular Targets for Chemoprevention*, Boca Raton, FL: CRC Press, 1992, pp. 223–243.
94. Plescia, O.J., Smith, A.H., and Grinwich, K. Subversion of immune system by tumor cells and the role of prostaglandins. *Proc. Natl. Acad. Sci. USA* 75: 1848–1851, 1975.
95. Goodwin, J.S. Immunologic effects of nonsteroidal anti-inflammatory drugs. *Am. J. Med.* 77: 7–15, 1984.
96. Cohen, S.M., Zenser, T.V., Murasaki, G., Fukushima, S., Mattammal, M.B., Rapp, N.S., and Davis, B.B. Aspirin inhibition of *N*-[4-(5-nitro-2-furyl)-2-thiazolyl] formamide-induced lesions of the urinary bladder correlated with inhibition of metabolism by bladder prostaglandin endoperoxide synthetase. *Cancer Res.* 41: 3355–3359, 1981.
97. Wild, D. and Degen, G.H. Prostaglandin H synthase-dependent mutagenic activation of heterocyclic aromatic amines of the IQ-type. *Carcinogenesis* 8: 541–545, 1987.
98. McCann, P.P., Bitonti, A.J., and Pegg, A.E. Inhibition of polyamine metabolism and the consequent effects on cell proliferation. *In:* Wattenberg, L., Lipkin, M., Boone, C.W., and Kelloff, G.J. (eds.), *Cancer Chemoprevention*, Boca Raton, FL: CRC Press, 1992, pp. 531–539.
99. Luk, G.D., Zhang, S.-Z., and Hamilton, S.R. Effects of timing of administration and dose of difluoromethylornithine on rat colonic carcinogenesis. *J. Natl. Cancer Inst.* 81: 421–427, 1989.
100. Ratko, T.A., Detrisac, C.J., Rao, C.V., Thomas, C.F., Kelloff, G.J., and Moon, R.C. Interspecies analysis of the chemopreventive efficacy of dietary α-difluoromethylornithine. *Anticancer Res.* 10: 67–72, 1990.
101. Kingsnorth, A.N., King, W.W.K., Diekema, K.A., McCann, P.P., Ross, J.S., and Malt, R.A. Inhibition of ornithine decarboxylase with 2–difluoromethylornithine: Reduced incidence of dimethylhydrazine-induced colon tumors in mice. *Cancer Res.* 43: 2545–2549, 1983.
102. Weeks, C.E., Herrmann, A.L., Nelson, F.R., and Slaga, T.J. α-Difluoromethylornithine, an irreversible inhibitor of ornithine decarboxylase, inhibits tumor promoter-induced polyamine accumulation and carcinogenesis in mouse skin. *Proc. Natl. Acad. Sci. USA* 79: 6028–6032, 1982.
103. Homma, Y., Ozono, S., Numata, I., Seidenfeld, J., and Oyasu, R. Inhibition of carcinogenesis by α-difluoromethylornithine in heterotopically transplanted rat urinary bladders. *Cancer Res.* 45: 648–652, 1985.

104. Homma, Y., Kakizoe, T., Samma, S., and Oyasu, R. Inhibition of *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine-induced rat urinary bladder carcinogenesis by α-difluoromethylornithine. *Cancer Res.* 47: 6176–6179, 1987.
105. Thompson, H.J., Herbst, E.J., Meeker, L.D., Minocha, R., Ronan, A.M., and Fite, R. Effect of D,L-alpha-difluoromethylornithine on murine mammary carcinogenesis. *Carcinogenesis* 5: 1649–1651, 1984.
106. Thompson, H.J., Meeker, L.D., Herbst, E.J., Ronan, A.M., and Minocha, R. Effect of concentration of D,L-2-difluoromethylornithine on murine mammary carcinogenesis. *Cancer Res.* 45: 1170-1173, 1985.
107. Wargovich, M.J., Eng, V.W.S., Newmark, H.L., and Bruce, W.R. Calcium ameliorates the toxic effect of deoxycholic acid on colonic epithelium. *Carcinogenesis* 4: 1205–1207, 1983.
108. Bird, R.P., Schneider, R., Stamp, D., and Bruce, W.R. Effect of dietary calcium and cholic acid on the proliferative indices of murine colonic epithelium. *Carcinogenesis* 7: 1657–1661, 1986.
109. Wargovich, M.J., Eng, V.W.S., and Newmark, H.L. Calcium inhibits the damaging and compensatory proliferative effects of fatty acids on mouse colon epithelium. *Cancer Lett.* 23: 253–258, 1984.
110. Buset, M., Lipkin, M., Winawer, S., Swaroop, S., and Friedman, E. Inhibition of human colonic epithelial cell proliferation *in vivo* and *in vitro* by calcium. *Cancer Res.* 46: 5426–5430, 1986.
111. Wargovich, M.J., Allnutt, D., Palmer, C., Anaya, P., and Stephens, L.C. Inhibition of the promotional phase of azoxymethane-induced colon carcinogenesis in the F344 rat by calcium lactate: Effect of simulating two human nutrient density levels. *Cancer Lett.* 53: 17–25, 1990.
112. Newmark, H.L., Wargovich, M.J., and Bruce, W.R. Colon cancer and dietary fat, phosphate, and calcium: A hypothesis. *J. Natl. Cancer Inst.* 72: 1323–1325, 1984.
113. Welberg, J.W.M., Kleibeuker, J.H., van der Meer, R., Mulder, N.H., and de Vries, E.G.E. Calcium and the prevention of colon cancer. *Scand. J. Gastroenterol.* 188: 52–59, 1991.
114. Ratko, T.A., Mehta, R.G., Detrisac, C.J., Kelloff, G.J., and Moon, R.C. Inhibition of rat mammary gland chemical carcinogenesis by dietary dehydroepiandrosterone or a fluorinated analogue of dehydroepiandrosterone. *Cancer Res.* 51: 481–486, 1991.
115. Ratko, T.A., Detrisac, C.J., Dinger, N.M., Thomas, C.F., Kelloff, G.J., and Moon, R.C. Chemopreventive efficacy of combined retinoid and tamoxifen treatment following surgical excision of a primary mammary cancer in female rats. *Cancer Res.* 49: 4472–4476, 1989.
116. Cohen, L.A., Epstein, M., Saa-Pabon, V., Meschter, C., and Zang, E. Interactions between 4-HPR and diet in NMU-induced mammary tumorigenesis. *Nutr. Cancer* 21: 271–283, 1994.
117. Moon, R.C., Steele, V.E., Kelloff, G.J., Thomas, C.F., Detrisac, C.J., Mehta, R.G., and Lubet, R.A. Chemoprevention of MNU-induced mammary tumorigenesis by diverse chemicals: Tamoxifen, toremifene, RU 16117, aminogluthethimide, and progesterone. *Anticancer Res.* 14: 889–894, 1994.
118. Lubet, R.A., Steele, V.E., Casebolt, T.L., Eto, I., Kelloff, G.J., and Grubbs, C.J. Chemopreventive effects of the aromatase inhibitors vorozole (R 83842) and 4-hydroxyandrostenedione in the methylnitrosourea (MNU)-induced mammary tumor model in Sprague-Dawley rats. *Carcinogenesis, in press.*
119. Bendich, A. and Olson, J.A. Biological actions of carotenoids. *FASEB J.* 3: 1927–1932, 1989.
120. Krinsky, N.I. β-Carotene: Functions. In: Spiller, G.A. and Scala, J. (eds.), *New Protective Roles For Selected Nutrients*, New York, NY: Alan R. Liss, Inc., 1989, pp. 1–15.
121. Rettura, G., Duttagupta, C., Listowsky, P., Levenson, S.M., and Seifter, E. Dimethylbenz(*a*)anthracene (DMBA)-induced tumors: Prevention by supplemental β-carotene (BC). *Fed. Proc.* 42: 786, abstract no. 2891, 1983.
122. Rettura, G., Levenson, S.M., and Seifter, E. 7,12-Dimethylbenz(*a*)anthracene (DMBA) carcinogenicity: Moderation by supplemental β-carotene (β-Car) and vitamin A (Vit A). *Fed. Proc.* 41: 67, abstract no. 11, 1982.
123. Nagasawa, H., Konishi, R., Sensui, N., Yamamoto, K., and Ben-Amotz, A. Inhibition by β-carotene-rich algae *Dunaliella* of spontaneous mammary tumourigenesis in mice. *Anticancer Res.* 9: 71–76, 1989.
124. Grubbs, C.J., Eto, I., Juliana, M.M., and Whitaker, L.M. Effect of canthaxanthin on chemically induced mammary carcinogenesis. *Oncology* 48: 239–245, 1991.
125. Jordan, V.C. and Murphy, C.S. Endocrine pharmacology of antiestrogens as antitumor agents. *Endocr. Rev.* 11: 578–610, 1990.
126. Nayfield, S.G., Karp, J.E., Ford, L.G., Dorr, F.A., and Kramer, B.S. Potential role of tamoxifen in prevention of breast cancer. *J. Natl. Cancer Inst.* 83: 1450–1459, 1991.
127. Kangas, L. Review of the pharmacological properties of toremifene. *J. Steroid Biochem. Mol. Biol.* 36: 191–195, 1990.
128. Raynaud, J.P., Azadian-Boulanger, G., Bouton, M.M., Colin, M.C., Faure, N., Fernand-Proulx, L., Gautray, J.P., Husson, J.M., Jolivet, A., Kelly, P., Labrie, F., Ojasoo, T., and Precigoux, G. RU 16117, an orally active estriol-like weak estrogen. *J. Steroid Biochem.* 20: 981–993, 1984.
129. Jordan, V.C. Effect of tamoxifen (ICI 46,474) on initiation and growth of DMBA-induced rat mammary carcinoma. *Eur. J. Cancer Clin. Oncol.* 12: 419–424, 1976.
130. Inoh, A., Kamiya, K., Fuji, Y., and Yokoro, K. Protective effects of progesterone and tamoxifen in estrogen-induced mammary carcinogenesis in ovariectomized W/Fu rats. *Jpn. J. Cancer Res.* 76: 699–704, 1985.
131. Gottardis, M.M. and Jordan, V.C. Antitumor actions of keoxifene and tamoxifen in the *N*-nitrosomethyl-

urea-induced rat mammary carcinoma model. *Cancer Res.* 47: 4020–4024, 1987.

132. Robinson, S.P., Mauel, D.A., and Jordan, V.C. Antitumor actions of toremifene in the 7,12-dimethylbenzanthracene (DMBA)-induced rat mammary tumor model. *Eur. J. Cancer Clin. Oncol.* 24: 1817–1821, 1988.
133. Kelly, P.A., Asselin, J., Caron, M.G., Raynaud, J.-P., and Labrie, F. High inhibitory activity of a new antiestrogen, RU 16117 (11α-methoxy ethinyl estradiol), on the development of dimethylbenz(*a*)anthracene-induced mammary tumors. *Cancer Res.* 37: 76–81, 1977.
134. Kelly, P.A., Asselin, J., Caron, M.G., Labrie, F., and Raynaud, J.-P. Potent inhibitory effect of a new antiestrogen (RU 16117) on the growth of 7,12-dimethylbenz[*a*]anthracene-induced rat mammary tumors. *J. Natl. Cancer Inst.* 58: 623–628, 1977.
135. Turcot-Lemay, L. and Kelly, P.A. Characterization of estradiol, progesterone, and prolactin receptors in nitrosomethylurea-induced mammary tumors and the effect of antiestrogen treatment on the development and growth of these tumors. *Cancer Res.* 40: 3232–3240, 1980.
136. Grubbs, C.J., Peckham, J.C., and McDonough, K.D. Effect of ovarian hormones on the induction of 1-methyl-1-nitrosourea-induced mammary cancer. *Carcinogenesis* 4: 495–497, 1983.
137. Grubbs, C.J., Farnell, D.R., Hill, D.L., and McDonough, K.C. Chemoprevention of *N*-nitroso-*N*-methylurea-induced mammary cancers by pretreatment with 17β-estradiol and progesterone. *J. Natl. Cancer Inst.* 74: 927–931, 1985.
138. Kellis, J.T., Jr., Nesnow, S., and Vickery, L.W. Inhibition of aromatase cytochrome P-450 (estrogen synthetase) by derivatives of α-naphthoflavone. *Biochem. Pharmacol.* 35: 2887–2891, 1986.
139. Cole, P.A. and Robinson, C.H. Mechanism and inhibition of cytochrome P-450 aromatase. *J. Med. Chem.* 33: 2933–2942, 1990.
140. De Coster R., Van Ginckel R., Wouters, W., Goeminne, N., Vanherck, W., and Byloos, M. Endocrine and antitumoral effects of R76713 in rats. *J. Enzym. Inhib.* 4: 159–167, 1990.
141. De Coster, R., Wouters, W., Bowden, C.R., Van den Bossche, H., Bruynseels, J., Tuman, R.W., van Ginckel, R., Snoeck, E., van Peer, A., and Janssen, P.A.J. New non-steroidal aromatase inhibitors: Focus on R76713. *J. Steroid Biochem. Mol. Biol.* 37: 335–341, 1990.
142. Bradfield, C.A. and Bjeldanes, L.F. Effect of dietary indole-3-carbinol on intestinal and hepatic monooxygenase, glutathione *S*-transferase and epoxide hydrolase activities in the rat. *Food. Chem. Toxicol.* 22: 977–982, 1984.
143. Michnovicz, J.J. and Bradlow, H.L. Altered estrogen metabolism and excretion in humans following consumption of indole-3-carbinol. *Nutr. Cancer* 16: 59–66, 1991.
144. Wattenberg, L.W. and Loub, W.D. Inhibition of polycyclic aromatic hydrocarbon-induced neoplasia by naturally occurring indoles. *Cancer Res.* 38: 1410–1413, 1978.
145. Schwartz, A.G. Inhibition of spontaneous breast cancer formation in female C3H A^{vy}/a mice by long-term treatment with dehydroepiandrosterone. *Cancer Res.* 39: 1129–1132, 1979.
146. Schwartz, A.G. and Tannen, R.H. Inhibition of 7,12-dimethylbenz[*a*]anthracene- and urethan-induced lung tumor formation in A/J mice by long-term treatment with dehydroepiandrosterone. *Carcinogenesis* 2: 1335–1337, 1981.
147. Schwartz, S.G., Hard, G.C., Pashko, L.L., Abou-Gharbia, M., and Swern, D. Dehydroepiandrosterone: An anti-obesity and anti-carcinogenic agent. *Nutr. Cancer* 3: 46-53, 1981.
148. Nyce, J.W., Magee, P.N., Hard, G.C., and Schwartz, A.G. Inhibition of 1,2-dimethylhydrazine-induced colon tumorigenesis in Balb/c mice by dehydroepiandrosterone. *Carcinogenesis* 5: 57–62, 1984.
149. Pashko, L.L., Rovito, R.J., Williams, J.R., Sobel, E.L., and Schwartz, A.G. Dehydroepiandrosterone (DHEA) and 3β-methylandrost-5-en-17-one: Inhibitors of 7,12-dimethylbenz[*a*]anthracene (DMBA)-initiated and 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-promoted skin papilloma formation in mice. *Carcinogenesis* 5: 463–466, 1984.
150. Pashko, L.L., Hard, G.C., Rovito, R.J., Williams, J.R., Sobel, E.L., and Schwartz, A.G. Inhibition of 7,12-dimethylbenz(*a*)anthracene-induced skin papillomas and carcinomas by dehydroepiandrosterone and 3β-methylandrost-5-en-17-one in mice. *Cancer Res.* 45: 164–166, 1985.
151. Moore, M.A., Thamavit, W., Tsuda, H., Sato, K., Ichihara, A., and Ito, N. Modifying influence of dehydroepiandrosterone on the development of dihydroxy-di-*N*-propylnitrosamine-initiated lesions in the thyroid, lung and liver of F344 rats. *Carcinogenesis* 7: 311–316, 1986.
152. Gordon, G.B., Ahantz, L.M., and Talalay, P. Modulation of growth, differentiation and carcinogenesis by dehydroepiandrosterone. *Adv. Enzyme Regul.* 26: 355–382, 1987.
153. Weber, E., Moore, M.A., and Bannasch, P. Phenotypic modulation of hepatocarcinogenesis and reduction in *N*-nitrosomorpholine-induced hemangiosarcoma and adrenal lesion development in Sprague-Dawley rats by dehydroepiandrosterone. *Carcinogenesis* 9: 1191–1195, 1988.
154. Ratko, T.A., Detrisac, C.J., Mehta, R.G., Kelloff, G.J., and Moon, R.C. Inhibition of rat mammary gland chemical carcinogenesis by dietary dehydroepiandrosterone or a fluorinated analogue of dehydroepiandrosterone. *Cancer Res.* 51: 481–486, 1991.
155. Schwartz, A.G., Lewbart, M.L., and Pashko, L.L. Inhibition of tumorigenesis by dehydroepiandrosterone and structural analogs. *In:* Wattenberg, L., Lipkin, M., Boone, C.W., and Kelloff, G.J. (eds.), *Cancer Chemoprevention*, Boca Raton, FL: CRC Press, 1992, pp. 205–226.

156. Schwartz, A.G. and Perantoni, A. Protective effect of dehydroepiandrosterone against aflatoxin B_1- and 7,12 dimethylbenz(*a*)anthracene-induced cytotoxicity and transformation in cultured cells. *Cancer Res.* 35: 2482–2487, 1975.
157. Feo, F., Pirisi, L., Pascale, R., Daino, L., Frassetto, S., Garcea, R., and Gaspa, L. Modulatory effect of glucose-6-phosphate dehydrogenase deficiency on benzo(*a*)pyrene toxicity and transforming activity for *in vitro* cultured human skin fibroblasts. *Cancer Res.* 44: 3419–3425, 1984.
158. Feo, F., Ruggiu, M.E., Lenzerini, L., Garcea, R., Daino, L., Frassetto, S., Addis, V., Gaspa, L., and Pascale, R. Benzo(*a*)pyrene metabolism by lymphocytes from normal individuals and individuals carrying the Mediterranean variant of glucose-6-phosphate dehydrogenase. *Int. J. Cancer* 39: 560–564, 1987.
159. Schwartz, A.G., Fairman, D.K., Polansky, M., Lewbart, M.L., and Pashko, L.L. Inhibition of 7,12-dimethylbenz(*a*)anthracene-initiated and 12-*O*-tetradecanoylphorbol-13-acetate-promoted skin papilloma formation in mice by dehydroepiandrosterone and two synthetic analogs. *Carcinogenesis* 10: 1809–1813, 1989.
160. Pashko, L.L., Schwartz, A.G., Abou-Gharbia, M., and Swern, D. Inhibition of DNA synthesis in mouse epidermis and breast epithelium by dehydroepiandrosterone and related steroids. *Carcinogenesis* 2: 717–721, 1981.
161. Pashko, L.L., Lewbart, M.L., and Schwartz, A.G. Inhibition of 12-*O*-tetradecanoylphorbol-13-acetate-promoted skin tumor formation by 16α-fluoro-5-androsten-17-one and its reversal by deoxyribonucleosides. *Carcinogenesis* 12: 2189–2192, 1991.
162. Knudsen, J.F. and Mahesh, V.B. Initiation of precocious sexual maturation in the immature rat treated with dehydroepiandrosterone. *Endocrinology* 97: 458–468, 1975.
163. Schwartz, A.G., Lewbart, M.L., and Pashko, L.L. Novel dehydroepiandrosterone analogues with enhanced biological activity and reduced side effects in mice and rats. *Cancer Res.* 48: 4817–4822, 1988.
164. Yamada, J., Sakuma, M., Ibeda, T., Fukuda, K., and Suga, T. Characteristics of dehydroepiandrosterone as a peroxisome proliferator. *Biochem. Biophys. Acta* 1092: 233–243, 1991.
165. van Duuren, B.L., Blazej, T., Goldschmidt, B.M., Katz, C., Melchionne, S., and Sivak, A. Cocarcinogenesis studies on mouse skin and inhibition of tumor induction. *J. Natl. Cancer Inst.* 46: 1039–1044, 1971.
166. van Duuren, B.L. and Goldschmidt, B.M. Cocarcinogenic and tumor-promoting agents in tobacco carcinogenesis. *J. Natl. Cancer Inst.* 56: 1237–1242, 1976.
167. Kato, R., Nakadate, T., Yamamoto, S., and Sugimura, T. Inhibition of 12-*O*-tetradecanoylphorbol-13-acetate-induced tumor promotion and ornithine decarboxylase activity by quercetin: Possible involvement of lipoxygenase inhibition. *Carcinogenesis* 4: 1301–1305, 1983.
168. Nishino, H., Iwashima, A., Fujiki, H., and Sugimura, T. Inhibition by quercetin of the promoting effect of teleocidin on skin papilloma formation in mice initiated with 7,12-dimethylbenz[*a*]anthracene. *Gann* 75: 113–116, 1984.
169. Nakadate, T., Yamamoto, S., Aizu, E., and Kato, R. Effects of flavonoids and antioxidants on 12-*O*-tetradecanoylphorbol-13-acetate-caused epidermal ornithine decarboxylase induction and tumor promotion in relation to lipoxygenase inhibition by these compounds. *Gann* 75: 214–222, 1984.
170. Chang, R.L., Huang, M.-T., Wood, A.W., Wong, C.-Q., Newmark, H.L., Yagi, H., Sayer, J.M., Jerina, D.M., and Conney, A.H. Effect of ellagic acid and hydroxylated flavonoids on the tumorigenicity of benzo[*a*]pyrene and (±)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene on mouse skin and in the newborn mouse. *Carcinogenesis* 6: 1127–1133, 1985.
171. Fujiki, H., Horiuchi, T., Yamashita, K., Hakii, H., Suganuma, M., and Nishino, H. Inhibiton of tumor promotion by flavonoids. *Prog. Clin. Biol. Res.* 213: 429–440, 1986.
172. Mukhtar, H., Das, M., Khan, W.A., Wang, Z.Y., Bik, D.P., and Bickers, D.R. Exceptional activity of tannic acid among naturally occurring plant phenols in protecting against 7,12-dimethylbenz(*a*)anthracene-, benzo(*a*)pyrene-, 3-methylcholanthrene-, and *N*-methyl-*N*-nitrosourea-induced skin tumorigenesis in mice. *Cancer Res.* 48: 2361–2365, 1988.
173. Verma, A.K., Johnson, J.A., Gould, M.N., and Tanner, M.A. Inhibition of 7,12-dimethylbenz(*a*)anthracene- and *N*-nitrosomethylurea-induced rat mammary cancer by dietary flavonol quercetin. *Cancer Res.* 48: 5754–5758, 1988.
174. Yasukawa, K., Takido, M., Takeuchi, M., and Nitta, K. Effect of flavonoids on tumor promoter's activity. *Prog. Clin. Biol. Res.* 280: 247–250, 1988.
175. McCormick, D.L., Bagg, B.J., and Hultin, T.A. Comparative activity of dietary or topical exposure to three retinoids in the promotion of skin tumor induction in mice. *Cancer Res.* 47: 5989–5993, 1987.
176. Nishino, H., Kitagawa, K., and Iwashima, A. Anti-tumor-promoting activity of glycyrrhetic acid in mouse skin tumor formation induced by 7,12-dimethylbenz[*a*]anthracene plus teleocidin. *Carcinogenesis* 5: 1529–1530, 1984.
177. Nishino, H., Yoshioka, K., Iwashima, A., Takizawa, H., Konishi, S., Okamoto, H., Okabe, H., Shibata, S., Fujiki, H., and Sugimura, T. Glycyrrhetic acid inhibits tumor-promoting activity of teleocidin and 12-*O*-tetradecanoyl-phorbol-13-acetate in two-stage mouse skin carcinogenesis. *Jpn. J. Cancer Res.* 77: 33–38, 1986.
178. Wang, Z.Y., Agarwal, R., Zhou, Z.C., Bickers, D.R., and Mukhtar, H. Inhibition of mutagenicity in *Salmonella typhimurium* and skin tumor initiating and tumor promoting activities in SENCAR mice by glycyrrhetinic acid: Comparison of 18α- and 18β-stereoisomers. *Carcinogenesis* 12: 187–192, 1991.

179. Moon, R.C., Detrisac, C.J., Thomas, C.F., and Kelloff, G.J. Chemoprevention of experimental bladder cancer. *J. Cell. Biochem.* 16 (Suppl. I): 134–138, 1992.
180. Grubbs, C.J., Juliana, M.M., Eto, I., Casebolt, T., Whitaker, L.M., Canfield, G.J., Manczak, M., Kelloff, G.J., and Steele, V.E. Chemoprevention by indomethacin of hydroxy(butyl)nitrosamine-induced urinary bladder tumors. *Anticancer Res.* 13: 33–36, 1993.
181. Klän, R., Knispel, H.H., and Meier, T. Acetylsalicylic acid inhibition of *N*-butyl-(4-hydroxybutyl)nitrosamine-induced bladder carcinogenesis in rats. *J. Cancer Res. Clin. Oncol.* 119: 482–485, 1993.
182. Moon, R.C., Rao, K.V.N., Detrisac, C.J., and Kelloff, G.J. Animal models for chemoprevention of respiratory cancer. *Monogr. Natl. Cancer Inst.* 13: 45–49, 1992.