

Meeting Report

Current Strategies of Cancer Chemoprevention: 13th Sapporo Cancer Seminar

The broad concept of chemoprevention applies to the prevention of clinical cancer by the administration of pharmaceuticals or dietary constituents. In recent years there has been a rapid expansion of basic research on mechanisms of chemoprevention, and more and more candidate compounds are entering clinical trials. It is therefore timely that the subject of the 13th Sapporo Cancer Seminar held on July 6-9, 1993, was "Current Strategies of Cancer Chemoprevention." The Seminar was organized by Drs. H. Fujiki, H. Kobayashi, L. W. Wattenberg, C. W. Boone, and G. J. Kelloff.

Chemoprevention by Minor Nonnutrient Constituents of the Diet

Dr. L. Wattenberg (University of Minnesota, Minneapolis, MN) described the growing awareness in recent years that dietary nonnutrient compounds can have extremely important effects on the consequences of exposure to carcinogens, drugs, and an assortment of other potentially toxic materials. He described examples of such compounds including tea tannins, flavonoids, terpenes, isothiocyanates, organosulfur compounds, protease inhibitors, and inositols (1). Some chemopreventives are blocking agents; *i.e.*, they prevent carcinogens from reaching or reacting with critical target sites on DNA. Others are suppressing agents; *i.e.*, they prevent evolution of the neoplastic process in cells which would otherwise become malignant (2).

Chemoprevention Program at The NCI,¹ Bethesda, MD

Dr. P. Greenwald (DCPC, NCI, Bethesda, MD) described six NCI-sponsored large-scale chemoprevention trials that are in progress. Four are using micronutrients, as follows: (a) β -carotene alone; (b) β -carotene and retinol; (c) β -carotene and vitamin E; and (d) in a study in China, various combinations of vitamins A, C, and E; zinc; riboflavin; niacin; molybdenum; selenium; and β -carotene. Two other trials are testing the efficacy of tamoxifen in preventing breast cancer and finasteride in preventing prostate cancer. The NCI has established a chemoprevention trials decision network to facilitate the development of novel chemopreventive agents.

Dr. C. W. Boone (CISB, DCPC, NCI, Bethesda, MD) reported that 22 drugs and 3 drug combinations have reached an advanced stage of development in the program of the CISB. The first generation of drugs, now in Phase II and Phase III clinical trials, are those described by Dr. Greenwald above. The second generation, in Phase I clinical trials, are four NSAIDs (piroxicam, ibuprofen, aspirin, and sulindac), 2-difluoromethylornithine, carbenoxolone, oltipraz (a dithiolthione), and the combination of 2-difluoromethylornithine with piroxicam. The third generation, showing efficacy in animal models and being tested for toxicity, are ellagic acid, phenethyl isothiocyanate, curcumin, perillyl alcohol, S-allylcysteine, N-acetylcysteine, fluasterone (16-fluorodehydroepiandrosterone), and the combinations 4-HPR plus oltipraz and 4-HPR plus tamoxifen. In planning for the continuous identification and development of new chemopreventive compounds, it has been found useful in practice to classify chemopreventives as

either antimutagenic or antimutagenic. Antioxidants, because of their similar mechanism of action, have been grouped separately as a third class. Antioxidants are both antimutagenic and antimutagenic.

Clinical Trials of Chemopreventive Agents

Head and Neck. Dr. W-K. Hong (University of Texas M. D. Anderson Cancer Center, Houston, TX) reviewed the successful use of 13-cRA against the development of upper aerodigestive tract neoplasia. In trials in human oral premalignancy, or leukoplakia, 13-cRA achieved a significant objective response rate of 67% (3). In an adjuvant trial to prevent second primary tumors in patients initially "cured" of head and neck squamous cell carcinoma, 13-cRA significantly reduced the high incidence of these usually fatal second malignancies (4). Moderate to severe side effects of 13-cRA include: skin dryness (63% *versus* 8% placebo); cheilitis (24% *versus* 2% placebo); and conjunctivitis (18% *versus* 8% placebo). Although largely reversible, this toxicity is unacceptable for many subjects. Regimens of 13-cRA that include an initial high dose followed by low dose maintenance, or maintenance with another less toxic retinoid, are under evaluation.

Dr. M. Saikawa (National Cancer Center Hospital East, Kashiwa-City, Japan) described plans for a randomized clinical trial of β -carotene in the prevention of second primary tumors of the head and neck. Male patients will be enrolled just after surgery for squamous cell carcinoma of the oral cavity, pharynx, or larynx and will receive either 30 mg β -carotene or placebo daily for 5 years. Subjects will be examined every 4-8 weeks for recurrence and/or second primary cancer. It will take 5 years to recruit 405 patients for the study and another 5 years to obtain final results.

Breast. Dr. A. Costa (Istituto Nazionale Tumori, Milan, Italy) reported progress on a randomized, placebo-controlled clinical trial of breast cancer chemoprevention with 200 mg of 4-HPR daily. Started in 1987, the cohort consists of women with a history of previous surgery for breast cancer who are being followed for a second primary cancer in the contralateral breast. By April 30, 1993, 1492 patients had been randomized to the 4-HPR group and 1474 had been randomized to the control group. A 5-year randomized, placebo-controlled trial has also begun on the efficacy of tamoxifen, 20 mg daily, in preventing breast cancer in healthy women. Enrollment is restricted to hysterectomized subjects, because the risk of endometrial cancer has not yet been completely assessed. As of April 30, 1993, 801 women, average age 51 years, were entered into the study.

Liver. Dr. Y. Muto (Gifu University School of Medicine, Gifu, Japan) described the beginning of a Phase II randomized, double-blind trial of an acyclic retinoid, E5166, to reduce the recurrence rate of hepatoma in cirrhotic patients with previously resected hepatomas. The risk of a new hepatoma in these patients is 20-40%/year. E5166 appears to be of low toxicity and prevents liver tumors in rats given 3'-methyl-4-dimethylamino-benzene as well as in C3H/HeNCrj mice that develop spontaneous hepatomas. E5166 modulates the gene expression and secretion of α -fetoprotein and albumin in cultured human hepatoma cells in the direction of normal differentiation.

Colon. Dr. D. Earnest (Arizona Cancer Center, Tucson, AZ) reviewed the potential chemopreventive mechanisms of NSAID. They have antiproliferative effects on tumor cells *in vitro* and prevent carcinogen- and radiation-induced intestinal cancer in rodents. In patients with familial adenomatous polyposis, sulindac promotes re-

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¹ The abbreviations used are: NCI, National Cancer Institute; NSAID, nonsteroidal antiinflammatory drug; 13-cRA, 13-*cis*-retinoic acid; 4-HPR, *N*-(4-hydroxyphenyl)-retinamide; E5166, 3,7,11,15-tetramethyl-2,4,6,10,14-hexadecapentaenoic acid; SCLC, small cell lung carcinoma; SEB, surrogate end point biomarker; DMBA, 7,12-dimethylbenz[*a*]anthracene; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; EGCG, epigallocatechin gallate; DCPC, Division of Cancer Prevention and Control; CISB, Chemoprevention Investigational Studies Branch.

gression and prevents recurrence of adenomatous colon polyps. At least three epidemiological studies have reported a protective effect of aspirin against colorectal cancer incidence and mortality (5–7). The precise mechanism(s) by which NSAIDs exert their chemopreventive effect is not yet clear. NSAIDs inhibit the cyclooxygenase of prostaglandin H synthase and the secondary activation of procarcinogens by the hydroperoxidase activity of the same enzyme. They also suppress the activity of other important intracellular enzymes such as phosphodiesterase, protein kinases, and folate-dependent enzymes. In higher concentrations, NSAIDs interfere with cell membrane-associated processes including G-protein-mediated signal transduction, transmembrane calcium flux, and cell-cell adhesion. NSAIDs also enhance immunological responses. The main negative aspect of NSAID treatment is the dose-related occurrence of erosive gastroduodenal mucosal injury and bleeding. Piroxicam at the relatively low dose of 7.5 mg once daily is being evaluated for testing in clinical trials.

Lung. Dr. T. Ohnoshi (Okayama University Medical School, Okayama, Japan) strongly emphasized the need for chemoprevention of second primary tumors in long-term survivors of SCLC. Development of second malignancy was evaluated in 261 SCLC patients who received intensive chemotherapy, with or without radiotherapy, between 1975 and 1990. The cumulative probability for the development of second malignancy was 1.2% at 2 years, 8.5% at 3 years, 8.5% at 5 years, 21% at 6 years, and 53% at 8 years. The risk of non-SCLC (6.8-fold), acute nonlymphocytic leukemia (54.1-fold), and esophageal cancer (7.7-fold) was particularly high.

Melanoma. Dr. K. Zanker (Institute of Immunology, University of Witten/Herdecke, Göttingen, Germany) presented preliminary data showing coumarins (1,2-dibenzopyrone and its derivatives) to be effective in preventing early recurrence of melanoma following therapy. Coumarins include several hundred substances of varying structural complexity, which have been isolated from various plants. Coumarins are chemopreventive in animal models (8) and have been tested previously for treatment of melanoma (9).

Multisite. Dr. J-Y. Li (Cancer Institute, Chinese Academy of Medical Sciences, Beijing, China) reported the results of two randomized, double-blind, placebo-controlled nutrition-intervention trials, one with 3,318 subjects with esophageal dysplasia lasting 6 years and another with 29,584 subjects from the general population lasting 5.25 years. In the esophageal dysplasia trial, multiple daily vitamin/mineral supplements did not produce significant reduction of dysplasia but did produce a remarkable reduction in senile eye nuclear cataracts (43%). In the general population trial, those receiving the combination of β -carotene, vitamin E, and selenomethionine exhibited significant ($P < 0.05$) mortality reduction of 9%, cancer mortality of 13%, and gastric cancer mortality of 21%. Patterns of reduction in cancer incidence resembled those for cancer mortality. Furthermore, the protective effect on senile eye nuclear cataract found in the dysplasia trial was confirmed (41%, $P < 0.001$). In addition, in the general population trial, dietary supplementation with the combination of riboflavin and niacin significantly reduced the incidence of esophageal cancer (relative risk, 0.85; 95% confidence interval, 0.73–0.99; $P < 0.038$).

Surrogate End Point Biomarkers

Dr. C. W. Boone (CISB, DCPC, NCI, Bethesda, MD) underscored the urgent need for SEBs which can be used as the end point in relatively short-term clinical trials of chemopreventive agents, as opposed to the currently used end point of cancer incidence reduction in trials which are time consuming (5–10 years), labor intensive (thousands of subjects), and forbiddingly costly (millions of dollars).

He described three potentially useful SEBs based on the morphological, chromosomal, and molecular changes of intraepithelial (*i.e.*, preinvasive) neoplasia, which is synonymous with the term dysplasia, measured with the assistance of computerized morphometric and photometric analysis (10). These are: (a) the proliferative index, measured by thymidine/5-bromodeoxyuridine uptake or proliferating cell nuclear antigen/MIB-1 antibody probes; (b) aneuploidy or aneuploidy, measured by DNA densitometry of Feulgen-stained nuclei or by fluorescent *in situ* antibody probes to specific chromosome segments; and (c) nuclear pleomorphism, calculated as the mean of the three variances obtained from computer measurements of nuclear area, nuclear shape, and nuclear stain uptake. The group of abnormal nuclear morphological changes that constitute dysplasia, a phase of intraepithelial neoplasia, is an integral part of the pathway to cancer and as such is already validated as a SEB (10). That nuclear dysplastic changes are valid SEBs is supported by the fact that removing adenomatous polyps of the colon, which are prototypic examples of dysplasia, results in a decrease in the incidence of colon cancer (10).

Dr. M. Lipkin (Memorial Sloan-Kettering Cancer Center, New York, NY) described the development of SEBs in the colorectum, based on measuring the rates and distributions of proliferating and differentiating cells in the colon crypts. Measurements of prostanoids and their synthetic enzymes are being analyzed in relation to the effect of chemopreventive drugs, calcium, and vitamin D.

Dr. S. Y. Kim (Chungnam National University, Daejeon, Korea) reviewed programs in Korea that provide for vaccination against the hepatitis B virus, modification of diet, and antismoking education aimed at preventing three common cancers, liver cancer, stomach cancer, and lung cancer. As in other countries, the development of chemopreventive agents in Korea is being greatly slowed because the use of cancer incidence reduction as the end point in clinical trials requires longer observation periods than standard Phase III trials. In response to the urgent need for SEBs to overcome this problem, Dr. Kim has developed an *in situ* hybridization assay for generalized chromosomal polysomy as a marker of premalignant conditions and plans to use the assay to detect individuals at high risk for developing stomach cancer, the most common malignancy in Korea.

Dr. Y. Yamamoto (Department of Reaction Chemistry, University of Tokyo, Tokyo, Japan) proposed using the ratio of ubiquinol to ubiquinone in plasma as a measure of oxidative stress to monitor patients under treatment with antioxidant chemopreventive agents. The assay uses high-performance liquid chromatography with an electrochemical detector. The fraction of reduced ubiquinol in total ubiquinones (ubiquinol plus ubiquinone) was 0.88 ± 0.10 in healthy humans subjects. By contrast, it was 0.13 ± 0.15 ($P < 0.005$ by Student's *t* test) in patients with liver cancer.

Chemopreventive Agents under Development

Retinobenzoic Acids. K. Shudo (Faculty of Pharmaceutical Sciences, University of Tokyo, Tokyo, Japan) described the chemopreventive properties of the retinobenzoic acids, a subclass of arotinoids, which consist of an alkylaryl group and benzoic acid coupled through NHCO, CONH, OCO, COO, COC=C, or other groups. Some retinobenzoic acids suppress cell growth without toxicity, inhibit neoplastic transformation *in vitro* of DMBA-TPA-treated cells, inhibit metastasis by B16-F10 cells, and normalize hyperkeratotic and hyperkeratinized skin. Retinobenzoic acids are under evaluation to see if they retain chemopreventive efficacy without the characteristic toxic side effects of retinoic acid, such as hyperlipidemia, hyperostosis, teratogenicity, and cutaneous dryness, itching, and erythema.

Carotenoids. Dr. H. Nishino (Department of Biochemistry, Kyoto Prefectural University of Medicine, Kyoto, Japan) found that palm

fruit carotene, which contains a mixture of α -, β -, and γ -carotenes, lycopene, and other carotenoids is more potent than β -carotene in preventing lung, liver, and skin carcinogenesis in animal models. In particular, α -carotene was approximately 10 times more potent than β -carotene. Lutein, lactucaxanthin, β -cryptoxanthin, zeaxanthin, and asthaxanthin also appear to be promising as chemopreventive agents.

Monoterpenes. Dr. M. N. Gould (Department of Human Oncology, University of Wisconsin, Madison, WI) showed that *d*-limonene and perillyl alcohol prevent a variety of organ-specific cancers in rodent models. Significantly, they cause complete regression of established rat mammary carcinomas without toxicity. Histopathological study of terpene-induced regressing mammary carcinomas suggests that a cytostatic redifferentiation process is associated with the regression. Monoterpene exposure results in both increased levels of enzymes that could block DMBA initiation of mammary cancer and inhibition of enzyme activities associated with the mevalonate/lipid metabolism pathway including protein prenyltransferases.

Diterpenes. Dr. Y. Nakatsu (Department of Pathology, University of Tokyo, Tokyo, Japan) has found that sarcophytol A, a cembrane (*sic*)-type diterpene found in lipid extracts of the soft coral *Sarcophyton glaucum*, exhibits chemopreventive activity in animal models of skin, liver, and intestinal carcinogenesis. When given in the postinitiation phase in the diet for 38 weeks, sarcophytol inhibited lung tumors induced in rats by BHP placed in the drinking water for 2 weeks (0.005%). Dr. A. Komori (Cancer Prevention Division, National Cancer Research Institute, Tokyo, Japan) reported that canventol, a synthetic analogue of sarcophytol A with a simpler structure, inhibited tumor promotion by okadaic acid in the mouse skin DMBA/okadaic acid model more strongly than sarcophytol A and inhibited isoprenylation in NIH-3T3 cells more strongly than sarcophytol A and *d*-limonene.

Phenolic Compounds from Green and Black Tea. A number of participants reported on the chemopreventive effect of phenolic compounds from green and black tea. Dr. T. Yamane (First Department of Surgery, Kyoto Prefectural University of Medicine, Kyoto, Japan) found that EGCG inhibited mouse duodenal adenocarcinoma induced by *N*-ethyl-*N'*-nitro-*N*-nitrosoguanidine ($P < 0.001$), and rat gastric adenocarcinoma induced by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine ($P < 0.05$). In the latter case, statistically significant reduction of the 5-bromodeoxyuridine labeling index of the gastric mucosa was produced by EGCG. Dr. M. Suganuma (Cancer Prevention Division, National Cancer Research Institute, Tokyo, Japan) reported that EGCG inhibited promotion with teleocidin or okadaic acid in the two-stage mouse skin model. EGCG also inhibited the growth of lung and mammary cancer cell lines and significantly inhibited the release of tumor necrosis factor α by cultured BALB/3T3 cells induced by okadaic acid. Dr. T. Negishi (Faculty of Pharmaceutical Sciences, Okayama University, Okayama, Japan) showed that EGCG was antimutagenic in the *Salmonella* assay, the FM3A cell culture assay, and the *Drosophila* spot test. Mutagenesis by 3-aminohydroxy-1,4-5H-pyrido[4,3-*b*]indole and benzo(*a*)pyrene 7,8-diol-9,10-epoxide in the two *in vitro* systems and by a nitrosamine in the *Drosophila* assay was suppressed by EGCG. Dr. T. Otsuka (Faculty of Medicine, Kyushu University, Fukuoka, Japan) found that EGCG induced apoptosis in six human leukemia cell lines, as shown by DNA laddering of multiples of an approximately 200-base pair subunit. Suppression of proliferation was first seen at 10 μ M EGCG and reached 50% inhibition at 50 μ M. According to Dr. S. Taniguchi (Department of Molecular and Cellular Biology, Kyushu University, Fukuoka, Japan), EGCG prevented lung metastases of B16-F10 cells in syngeneic mice. Dr. H. Nishida (Department of Medicine, Gifu University School of Medicine, Gifu, Japan) found that EGCG inhibited spontaneous hepatoma in C3H/HeCCrj mice. Dr. D. A. Ballentine (Thomas J. Lipton

Company, Englewood Cliffs, NJ) described his recently developed methods for obtaining pure fractions of catechins, flavonols, flavonol glucosides, and theaflavins from green and black tea, and some preliminary results of pharmacokinetic studies on green tea catechins. Dr. C. W. Boone (CISB, DCPC, NCI, Bethesda, MD) reviewed research in America on green and black tea phenolics by investigators who were unable to attend the meeting, as follows. Drs. A. Conney and H. Mukhtar and their associates have found that p.o. administration of extracts of green tea phenolics, containing principally EGCG, inhibits both initiation and promotion stages of carcinogenesis in mouse skin (carcinogen/promoter: DMBA/TPA, UV light/TPA, DMBA/UV light, DMBA/teleocidin, DMBA/okadaic acid), mouse lung [induced by *N,N*-diethylnitrosamine, 4-(methylnitrosamino)-1-(3-pyridyl)-1- β -naphthol] and rat esophagus (induced by *N*-nitrosobenzylmethylamine) (12, 13). Green tea phenolics are also chemopreventive in animal models (14). Most importantly, green tea phenolics in the drinking water of mice produced partial regression; and in 4% of mice, complete regression of already established papillomas induced by DMBA or UV light (15). Mukhtar *et al.* (16) have shown that green tea phenolic fractions produce *in vitro* inhibition of cytochrome P-450 mixed function oxygenases, enhancement of the phase II enzymes glutathione-S-transferase and quinone reductase, and enhancement of the antioxidant enzymes glutathione peroxidase and catalase. These actions of green tea phenolic compounds may be related to the mechanism by which they are chemopreventive.

Arachidonic Acid Cascade Inhibitors. Dr. S. Yamamoto (Department of Pharmacology, School of Medicine, Keio University, Tokyo, Japan) found that topical application of inhibitors of phospholipase A₂ or lipoxygenase prevented two-stage mouse skin carcinogenesis promoted by either TPA or 7-bromomethylbenzo(*a*)-anthracene, a non-TPA type of promoter. The degree of inhibition of lipoxygenase by various inhibitors paralleled the degree of tumor inhibition. On the other hand, the effect of indomethacin, a cyclooxygenase inhibitor, on tumor promotion is known to be variable. No lipoxygenase inhibitor has been shown to be effective by p.o. administration. However, recently a novel p.o. effective lipoxygenase inhibitor, 11-[[5-(3-methoxy-4-ethoxycarbonyloxyphenyl)-2,4-pentadienyl]aminoethyl-4-diphenylmethoxy]pyridine, was shown to effectively prevent skin tumor formation promoted either by TPA or non-TPA-type promoters. Oral 11-[[5-(3-methoxy-4-ethoxycarbonyloxyphenyl)-2,4-pentadienyl]aminoethyl-4-diphenylmethoxy]pyridine also prevented complete skin carcinogenesis caused by repeated weekly treatments with benzo(*a*)pyrene.

Inducers of Radical Scavenger Proteins. Dr. N. Takeichi (Laboratory of Cell Biology) and Dr. M. Hosokawa (Laboratory of Pathology, Cancer Institute, Hokkaido University School of Medicine, Sapporo, Japan) described the remarkable phenomenon that the tumorigenicity and metastatic capability of mouse fibrosarcoma cell line QR-32 could be increased by incubating the QR-32 cells with host inflammatory cells, including activated macrophages. The authors postulated that the mechanism of the phenomenon was that reactive oxygen species (superoxide, peroxide, and hydroxyl free radical) produced by the host immune effector cells caused structural changes in the QR-32 cell line which formed the basis for increased tumorigenicity and metastatic capability. When given to animals, PSK, a protein-bound polysaccharide mixture, stimulates the free radical scavenger proteins Mn-superoxide dismutase, catalase, and glutathione peroxidase. Bismuth subnitrate p.o. induces the protein metallothionein, a scavenger for superoxide, peroxide, and hydroxyl free radical. Both PSK and bismuth subnitrate *in vitro* produced significant inhibition of the induction of tumorigenic and metastatic phenotypic changes in the QR-32 cells.

PSK. Dr. H. Kobayashi (Sapporo Cancer Seminar Foundation, Sapporo, Japan) further discussed the chemopreventive properties of PSK mixture obtained from cultured mycelia of the CM-101 strain of *Coriolus versicolor* belonging to basidiomycetes. PSK is a biological response modifier capable of exhibiting diverse biological activities. In Japan, this agent has been given p.o. to postoperative cancer patients. PSK reduced the incidence of tumor and/or prolonged the survival period in chemical carcinogen-induced, radiation-induced, and spontaneous animal cancer models (17). PSK did not interact with or inhibit drug-metabolizing enzymes and had no effect on mutagenicity in the Ames test. On the other hand, this agent scavenged active oxygen through the induction of Mn-superoxide dismutase, prevented the increase in frequency of anticancer agent-induced sister chromatid exchange, and suppressed fetal deformation induced by transplacental injection of a teratogen, suggesting an effect on the initiation or promotion phase of carcinogenesis. PSK also regulated cytokine production and enhanced the antitumor activity of effector cells such as killer T-cells and natural killer cells. Thus, this agent seems to act at multiple steps during carcinogenesis rather than a particular step. The main mechanism may be radical trapping, preventive effects against chromosome injury, and immunomodulative effects on cytokine production and effector cell function.

Designer Foods. Dr. H. Pierson (Preventive Nutrition Consultants, Inc., Woodinville, WA) presented the concept that naturally occurring phytochemicals in edible plant foods known to have chemopreventive activity in animals could be purposely formulated into a food product and studied *in toto* in humans. He proposed that blood levels of absorbed constituents could be correlated with the degree of modulation of important metabolic processes involving steroid hormones, prostaglandins, and xenobiotic detoxification.

Aberrant Crypt Bioassay. Dr. M. Wargovich (University of Texas M. D. Anderson Cancer Center, Houston, TX) described a highly useful animal screening assay for chemopreventive agents involving determining the growth rate of aberrant crypt foci in rats after the administration of 1,2-dimethylhydrazine. The assay requires only 4–8 weeks to perform and correlates closely with long-term chemoprevention assays in rodents. To date, over 60 agents have been screened. In particular, the bioassay permits comparison of the efficacy of different NSAIDs. The assay is being evaluated as a system to develop SEBs in the colorectum (18).

Comments and Recommendations for Future Research. The presentation at this meeting demonstrates the rapidly increasing numbers and varieties of promising cancer chemopreventive agents that exist and the increasing information that is being obtained concerning their mechanisms of action. Along with continued efforts at finding additional compounds with high efficacy and minimal toxicity, there is a need to expedite testing procedures in order to bridge the gap between agent identification and definitive clinical trials. There is a particularly urgent need for the development of shorter, more precise, and quality-controlled animal assays to measure chemopreventive efficacy so that drug development may proceed more efficiently on the many available lead compounds and their analogs. In addition to single agents, the use of defined and reproducible mixtures of chemopreventive substances derived from natural sources is becoming desirable. Examples of such mixtures are phenolic compounds from green and black tea, or of organic selenium compounds from yeast grown in high selenium substrate. In these instances, it is important for the Food and Drug Administration to develop clear guidelines for the preparation and testing of such mixtures. Finally, research emphasis should continue in the direction of developing standardized, precise, quality-controlled surrogate endpoint biomarkers for use in

short-term Phase II clinical intervention trials of chemopreventive agents.

There is uniformity of opinion that prevention is the best means of dealing with the cancer problem. The information provided at this meeting provided a further stimulus toward achieving this goal.

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