Cancer Chemoprevention Part 1: Retinoids and Carotenoids and Other Classic Antioxidants


Cancer chemoprevention is the use of specific natural or synthetic substances with the objective of reversing, suppressing, or preventing carcinogenic progression to invasive cancer. Currently, numerous chemopreventive agents are in various stages of development and testing. Part 1 of this two-part series provides an overview of issues unique to chemoprevention trials, including the use of surrogate biomarkers as end points. This is followed by a discussion of the retinoids, such as all-trans-retinoic acid (ATRA [Vesanoid]), 9-cis-retinoic acid (9cRA), and isotretinoin (Accutane), and the carotenoids (eg, beta-carotene and lycopene) and other "classic" antioxidants (eg, vitamins E and C and selenium). Research on these agents will be delineated by disease site when applicable. Part 2, which will appear in next month’s issue, will focus on hormonally mediated chemopreventive agents, such as tamoxifen (Nolvadex), finasteride (Proscar), oral contraceptives, and dehydroepiandrosterone (DHEA). Part 2 also will cover nonantioxidant natural agents, such as calcium, the polyphenols, the isothiocyanates, and genistein; nonsteroidal anti-inflammatory drugs (NSAIDS), such as celecoxib, sulindac sulfone, and aspirin; difluoro-methylornithine (DFMO [Efornithine]); oltipraz; and N-acetylcysteine. [ONCOLOGY(11):1643-1658, 1998]

Introduction

Cancer chemoprevention is defined as the use of chemical agents to suppress or reverse carcinogenesis to prevent the development of invasive cancer.[1,2] Two basic concepts underlie this approach to cancer control: the multistep nature of cancer development and field carcinogenesis.

The development of cancer occurs over years and involves multiple genetic and phenotypic alterations that lead to invasive cancer. Chemoprevention is based on the premise that intervention is possible during the many steps of this process.[2]

Based on animal model studies, carcinogenesis has been broadly divided into three phases: initiation, promotion, and progression. In initiation, a carcinogen interacts with DNA, producing a fixed mutation. The specific molecular change depends on the carcinogen and can be influenced by a number of factors, including the rate and type of carcinogenic metabolism and the response of the DNA repair function.

During promotion, the initiated cells proliferate. This stage occurs over a long period and can be altered by agents that affect growth rates.[3]

Progression is the phase between a premalignant lesion and the development of invasive cancer. During this stage, genetic and phenotypic changes occur, with the rate of progression based on the rate of genetic mutation and cell proliferation.[1-8] Studies of molecular progression in colon cancer support this model of carcinogenesis, which involves a series of acquired genetic changes.[9]

Field carcinogenesis is the concept that, in patients at risk, extensive, multifocal, genetically distinct premalignant and malignant lesions can occur within the whole carcinogen-exposed region. The classic example is exposure of the upper aerodigestive tract and lungs to the carcinogenic effects of tobacco.[10] The finding of one neoplasm in the exposed area provides evidence for the presence of multiple premalignant lesions of independent origin.[11] In this setting, lesion-specific therapy is insufficient; interventions that prevent the promotion and progression of unrecognized lesions are needed.

Issues Relevant to Prevention Trials

Study Design

Chemopreventive strategies can be applied to the general population or to high-risk groups.[11-13] For use as a chemopreventive agent among the general population, a compound must have minimal or no toxicity. Agents that show promise for this purpose include dietary constituents or their
analogs, as well as medicinals, such as nonsteroidal anti-inflammatory drugs (NSAIDs).[11-13] High-risk individuals include those who have a genetic predisposition to cancer, prior cancer diagnosis, history of a significant exposure to a carcinogen, or a histology that indicates an elevated likelihood of developing cancer. Because of their increased risk, some toxicity may be acceptable in these populations.

In addition, subjects at high risk are ideal subjects for clinical trials of chemopreventive agents because their increased incidence rates allow for smaller study sample sizes.[11,14,15] For example, selection of participants for studies of breast cancer chemoprevention generally relies on the identification of high-risk proliferative breast histology or epidemiologic factors known to increase a woman's risk of developing breast cancer. In general, women with a ≥ 20% lifetime risk of developing breast cancer are considered to be good candidates for participation in chemoprevention trials.[16]

Chemoprevention trials administer specific natural or synthetic substances with the objective of reversing, suppressing, or preventing carcinogenic progression to invasive cancers. In preclinical research, the efficacy and toxicity of a chemopreventive agent are assessed via in vitro cell screening systems and in vivo assays using animal models.[11-15]

Designs for phase I-III chemoprevention trials are based on similar principles as chemotherapeutic trials. A number of issues unique to chemoprevention exist, however. Whereas phase I chemotherapy trials identify maximum tolerable doses in patients with refractory cancer, phase I chemoprevention trials establish safe doses with minimal toxicity for relatively healthy subjects.[11,14,15]

**Study End Points: Surrogate End Point Biomarkers**

After establishing the dose level with the optimal chemopreventive toxicity profile, phase II clinical trials evaluate biological efficacy in a larger group of patients at high risk for specific cancers and provide data that characterize dose, safety, and toxicity in the selected population. The primary end points of phase II trials are biological indices of neoplasia, based on clinical, histologic, genetic, biochemical, proliferative, or differentiation-related properties, that can be used to estimate the potential for neoplastic progression to cancer and to determine the effect of the chemopreventive agent being tested on these indices.[11,14,15] These biological indices are referred to as surrogate, or intermediate, end point biomarkers.

Phase IIa trials are feasibility studies of surrogate end point biomarkers and can include dose de-escalation studies to determine the lowest, least toxic drug dose that retains biological activity. In phase IIb trials, preliminary surrogate end point biomarkers are confirmed by definitive randomized study of treatment and control arms.

After short-term activity is established, phase III trials are conducted to establish long-term efficacy in reducing cancer incidence. Phase III trials can require thousands of subjects and 5 to 10 years to complete. Innovative strategies, such as factorial designs and the use of a vanguard cohort, have been developed to maximize the use of limited resources.[11,14,15]

Cancer incidence is the obvious end point of a trial investigating a chemopreventive agent. The low incidence of cancer, however, even in high-risk populations, necessitates lengthy studies with thousands of patients, entailing tremendous expense.

Identification and validation of surrogate end point biomarkers is vital to prevention research; eventually, surrogate biomarkers may replace cancer incidence as end points in large-scale clinical trials. With the development of valid surrogate end point biomarkers, fewer subjects will be required for the study to achieve the desired level of statistical power, and interventions can be evaluated over a shorter period than is possible when cancer is used as the end point.[11,14,15]

**Characteristics of the Ideal Surrogate Biomarker**—A valid surrogate biomarker should be on the causal pathway of cancer, and not simply an associated change. It should be expressed differently in normal than in high-risk premalignant sites; change its pattern and/or degree of expression in correlation with the stage of carcinogenesis; have a low rate of spontaneous change; and be technically and logistically feasible to measure.[11,14]

It should be possible to modulate a surrogate end point biomarker by chemopreventive agents in such as way as to lead to a decrease in the incidence of cancer. Thus, surrogate end point biomarkers differ from susceptibility markers, such as certain genetic polymorphisms and mutagen sensitivity. Finally, surrogate biomarkers should have known sensitivity, specificity, and positive predictive value for the development of cancer.[11,14,16]

The best-studied biomarkers are nonspecific indicators of genotoxicity and cell proliferation. Types of biomarkers include clinical and histologic parameters, genetic markers, proliferation markers, biochemical indicators, and differentiation markers.[4-6,11,14-17]
Surrogate End Point Biomarkers Under Study—Surrogate end point biomarkers can include markers of certain site-specific changes, such as the premalignant lesions listed below, or markers of general cellular/molecular changes, such as altered differentiation and proliferation, that can occur at many sites. Site-specific surrogate end point biomarkers (premalignant changes) currently under study include ductal carcinoma in situ (breast), prostatic intraepithelial neoplasia (prostate), adenomas and aberrant crypts (colon), papillomas (bladder), cervical intraepithelial neoplasia and human papillomavirus (HPV; cervix), leukoplakia (oral cavity), and squamous metaplasia (eg, larynx and lung). DNA ploidy, growth factor receptors (eg, epidermal growth factor receptor [EGFR]), oncogene expression, loss of heterozygosity, quantitative morphometric features, and proliferation, differentiation, and apoptosis markers are examples of general surrogate biomarkers (biomarkers of cellular/molecular changes) currently under study.[11,14,16,17]

Classification of Chemopreventive Agents

Chemoprevention aims to directly modulate specific steps in the carcinogenic process, block mutagenic carcinogens, prevent DNA damage by free radicals, suppress epithelial cell hyperproliferation, and/or modulate epithelial cell differentiation and apoptosis (programmed cell death). Because the mechanisms of action of current chemopreventive agents are not well understood, there is no widely accepted system for classifying these agents.[11]

One proposed classification system uses two major groups: blocking agents and suppressing agents.[12] Blocking agents prevent cancer-producing compounds from reaching or reacting with critical target sites in the tissues. Blocking agents may prevent carcinogen activation, enhance detoxification of carcinogenic agents, or trap cancer-producing compounds before they reach or react with target sites in tissues.

Suppressing agents prevent the evolution of the neoplastic process in cells already altered by carcinogenic stimuli. The mechanisms of action of these agents are poorly defined.[12] Some act by producing differentiation; others specifically counteract the consequences of genotoxic events, in particular, oncogene activation; and still others selectively inhibit the proliferation of neoplastic cells.

A number of substances can be classified as both blocking and suppressing agents. In addition, the mechanisms of many chemopreventive agents have not been determined (Table 1).[11-13] Currently, numerous chemopreventive agents are in various stages of development. This variation in research phase and the need for additional work elucidating mechanisms of action make it difficult to design a unifying classification system for these agents.

Some agents are for general cancer chemoprevention, showing activity in many sites (eg, retinoids). Other agents are far more specific, having site-specific molecular targets—eg, selective estrogen receptor modulators (SERMs), which affect targets in the estrogen-signaling pathway and show activity in the breast; and finasteride (Proscar), which affects 5-alpha-reductase in the andro-gen-signaling pathway and is being tested in the prostate (Table 1 and Table 2).[18]

Multiple epidemiologic studies conducted during the 1980s noted a lower risk of cancer among people who had increased fruit and vegetable intake. With the development of the theory that the antioxidant vitamins in fruits and vegetables prevent carcinogenesis by interfering with oxidative damage to DNA, a number of large-scale, randomized trials of beta-carotene and other "classic antioxidants" were conducted.[2,3,19]

The retinoids have also been extensively studied, with significant strides made in basic research, as well as randomized clinical trials in a number of disease sites. In addition to the clinically well-studied agents discussed above, a number of novel agents are undergoing preclinical study.

Part 1 of this two-part series on cancer chemoprevention will discuss the retinoids and carotenoids and other classic antioxidants as a group, with delineation of research by disease site when applicable.

Part 2 of this article, which will appear in next month’s issue, will focus on hormonally mediated chemopreventive agents, such as tamoxifen (Nolvadex), finasteride, oral contraceptives, and dehydroepiandrosterone (DHEA). A number of these agents have site-specific mechanisms of action and will be discussed as such. Part 2 also will cover other dietary constituents, such as calcium, the polyphenols, the isothiocyanates, and genistein, as well as the NSAIDS, difluoromethylornithine (DFMO [Efflornithine]), oltipraz, and N-acetylcysteine (Table 2).

Retinoids and Carotenoids and Other Classic Antioxidants

Carotenoids constitute a class of over 600 natural compounds occurring predominantly in fruits and vegetables. Some carotenoids, such as beta-carotene, are provitamin A compounds that can be
converted into vitamin A in vivo. Retinoids constitute a class of over 2,000 agents, which include vitamin A and its natural (eg, retinyl esters) and synthetic (eg, fenretinide, targretin) analogs.[2] The best-studied retinoids, 13-cis-retinoic acid (13cRA, isotretinoin [Accutane]), 9-cis-retinoic acid (9cRA), and all-trans-retinoic acid (ATRA [Vesanoid]), occur endogenously at very low plasma levels and have been synthesized and administered at pharmacologic doses.[2,11] Although retinoids and carotenoids can share certain properties, they have distinct mechanisms of action and very different biological, pharmacologic, and clinical effects.

**Carotenoid Biology**

The mechanisms underlying the biological actions of carotenoids are unclear.[2,20] Their reported biological actions include antioxidant activity and immunoenhancement. Many carotenoids can quench singlet oxygen through a physical reaction in which the energy of the excited oxygen is transferred to the carotenoid.

In addition to their effects on singlet oxygen, carotenoids are also thought to suppress oxygen-free radicals. Oxygen-free radicals are believed to have a role in carcinogenesis; thus, there is interest in antioxidant compounds and their activity as a mechanism for cancer prevention.[2,20-22] It is unclear whether antioxidant activity is responsible for the chemopreventive effects of carotenoids observed in numerous animal models, however.

Although initially beta-carotene was thought to exert antioxidant effects (as do other carotenoids) potentially suitable for chemoprevention, subsequent basic study has shown that beta-carotene can have pro-oxidant effects under high pressures and oxidative stress, such as occur in the lungs of smokers.[23,24] This finding may help explain the significantly increased risk of lung cancer that was associated with beta-carotene in current smokers involved in the Alpha-Tocopherol/Beta-Carotene (ATBC) Cancer Prevention Study and Beta-Carotene and Retinol Efficacy Trial (CARET), which are discussed below.

The earlier focus on beta-carotene in clinical trials was based on practical considerations; it had been the only commercially available carotenoid for which human data supported safety.

Other carotenoids, such as lycopene and alpha-carotene, have shown chemopreventive activity in animal models.[2,25-27] Recent work demonstrates that lycopene is a more active inhibitor of human cancer cell proliferation than is beta-carotene. In vitro, lycopene has been shown to be the most efficient quencher of singlet oxygen among the carotenoids. Investigators have also shown lycopene to inhibit the proliferation of breast, lung, and endometrial human cancer cells in culture.

Other research suggests that lycopene may suppress growth factors that stimulate cellular proliferation.[26] This mechanism has considerable potential in a number of human cancers.

**Classic Antioxidant Biology**

The anticarcinogenic properties of a number of agents, including vitamin C, vitamin E, and selenium, are thought to derive from their high antioxidant capacity. This is postulated to protect DNA from oxidative damage and potential genotypic mutation events associated with cancer initiation and promotion. The most potent antioxidant in the vitamin E group, alpha-tocopherol, functions as a major free-oxygen radical scavenger. In addition, in vitro and animal model studies support the anticarcinogenic activity of alpha-tocopherol.[25]

A substantial body of literature has documented the cancer-preventing potential of the essential trace element selenium in animal models. Several hypotheses have been proposed to explain selenium's antitumorigenic activity. These include protection against oxidative damage through glutathione peroxidase, alterations in carcinogen metabolism, effects on the endocrine and immune systems, production of cytotoxic selenium metabolites, inhibition of protein synthesis, inhibition of specific enzymes, and stimulation of apoptosis.[2,28]

**Retinoid Biology**

Natural vitamin A (retinol) and its esters and synthetic analogs have the potential to inhibit or reverse the process of carcinogenesis.[1,2,11] They have been shown to be effective in a wide range of in vivo experimental systems, including skin, bladder, lung, breast, and oral carcinogenesis.[2-8,11,29,30]

Numerous retinoids have been used alone or in combination for the treatment of various human cancers, such as basal cell carcinoma, squamous cell carcinoma of the skin, cervical cancer, melanoma, dysplastic nevus syndrome, cutaneous T-cell lymphoma, acute promyelocytic leukemia, lung carcinoma, breast carcinoma, ovarian carcinoma, bladder carcinoma, renal cell carcinoma, and squamous cell carcinoma of the head and neck.[2,11,31]

Retinoids control normal cell growth, differentiation, and apoptosis during embryonic development and within epithelial tissues in later life.[32] Their effects are mediated by the complex interactions of their nuclear receptors.[33] Nuclear receptors for retinoids belong to the superfamily of receptors...
that mediate the effects of many compounds, including steroid and thyroid hormones, vitamin D, prostaglandins, and drugs that activate peroxisomal proliferation.[34]

There are two major classes of retinoid nuclear receptors: retinoic acid receptors (RARs) and retinoid-X-receptors (RXRs). Subtypes (alpha, beta, and gamma) have been identified within each class, and each subtype has multiple isoforms, leading to great biological diversity.[1,2,5,11,29-36] Individual RARs and RXRs appear to be expressed differently in different tissues and to have distinct biological functions. The receptors are involved in regulating transcription of specific genes, which, in turn, regulate cell differentiation, proliferation, and loss.[1,2,31]

The relationship between variations in receptor expression by organ site and responsiveness to chemopreventive agents is currently under study. The mechanisms by which the retinoids exert their chemopreventive effects may encompass a wide range of pathways. For example, in vitro work has shown limited activity of retinoic acid derivatives in ovarian cancer cell lines and preparations of ovarian cancer cells obtained from ascitic fluid. However, a significant synergistic inhibition of proliferation was demonstrated when transforming growth factor-beta (TGF-beta) was combined with ATRA. As TGF-beta is produced by normal ovarian surface epithelium and by underlying stromal cells, retinoic acid derivatives and inducers of TGF-beta may have in vivo chemopreventive activity.[37]

Natural vitamin A and its esters and the retinoic acid isomers, ATRA, 9cRA and 13cRA, currently are the most widely clinically tested retinoids. These naturally occurring retinoids tend to be in vivo pan-activators of receptors (non-receptor specific). Since these retinoic acids are readily interconverted in vivo, each can activate a wide spectrum of retinoid receptors, signaling pathways, and biological effects.

Current systemic therapy with these agents is limited by the substantial toxicities that result from activation of multiple signaling pathways. These toxicities involve numerous systems, including the skin and mucosae (dryness, desquamation, peeling, pruritus, dermatitis, cutaneous photosensitivity), liver (reversible elevations of hepatic enzymes), skeleton (ligament calcification, skeletal hyperostosis), central nervous system (headache), and reversible abnormalities in serum lipids.[30,36]

A major focus of retinoid drug development for chemoprevention involves retinoic-acid-receptor (RAR or RXR)-selective retinoids and their development through molecular targeting approaches.[18,38] Primary molecular targets (and related examples of selective retinoids) currently include pan-agonist RARs (LGD1550), RAR-alpha (AM80), RAR-beta (CD2317), RAR-gamma (CD437), and pan-agonist RARs and RXRs (9cRA). Selective molecular targeting is designed to develop retinoids with higher therapeutic indices, ie, greater activity and/or fewer toxic effects.

Studies in Multiple Disease Sites

Observational Data

Epidemiologic and experimental data support an association between increased dietary intake of yellow-green vegetables and fresh fruits that are rich in antioxidant vitamins and protection against lung, stomach, pancreatic, liver, colon, breast, cervical, and prostate cancer. Dietary and blood-based studies have confirmed these findings for specific nutrients.[25,39] Studies of Vitamin A and Beta-Carotene--Whereas most dietary intake studies distinguish intake of vitamin A from intake of beta-carotene and retinol, early prospective studies of diet and cancer related a decrease in lung cancer risk to total vitamin A intake.[21,23,40] For example, the Nurses' Health Study, which prospectively followed a cohort of more than 120,000 US women, found a statistically significant decrease in cancer risk among women with the highest intake of total vitamin A.[41]

Although the observational evidence is not entirely consistent, many prospective investigations have shown an inverse association between dietary intake or blood levels of beta-carotene and subsequent cancer risk. The largest study from Japan, with 250,000 subjects and 17 years of follow-up, demonstrated a statistically significant inverse relationship between beta-carotene and cancer risk.[42] Other dietary studies have reported significant inverse associations between beta-carotene intake and cancer risk. Two studies found decreased risks of lung and prostate cancer among those with high intake of vegetables and fruits rich in both beta-carotene and vitamin C.[19] In contrast, six prospective cohort studies found no significant association between dietary beta-carotene intake and subsequent overall cancer incidence or incidence of cancers of the colon and rectum, prostate, breast, lung, and pancreas.[2,19,21,41]

To overcome some of the lack of reliability of dietary studies, a number of prospective, blood-based
studies, which measure serum or plasma levels of micronutrients, have also been completed. Six of these studies reported lower cancer risks for those in the upper category of serum or plasma beta-carotene, with the most consistent pattern seen in relation to lung malignancies. In contrast, eight blood-based studies found no statistically significant association between beta-carotene levels and cancer risk.[2,19,21]

**Studies of Other Antioxidants**—Epidemiologic studies of the other classic antioxidants have also been completed. Studies, including the Health Professionals Follow-up Study, have linked reductions in lung, bladder, and prostate cancer to increased lycopene intake.[25,26,43] Epidemiologic work has provided some evidence to support an inverse association between vitamin E and overall cancer mortality.[44] Decreased levels of plasma alpha-tocopherol in epithelial tumors of the reproductive organs have been reported.[25]

In a study of environmental selenium levels and county levels of cancer mortality, total cancer mortality and mortality from cancers of the lung, colon and rectum, bladder, esophagus, pancreas, breast, ovary, and cervix were significantly lower in counties with intermediate or high selenium levels, as compared with counties with low selenium levels.[45] Various prospective and retrospective epidemiologic studies have both supported and refuted the association between selenium intake and cancer.[45-47] Using data from the Nurse's Health Study, researchers found no significant inverse association between selenium intake (as reflected in toenail selenium levels) and the risk of various cancers, including breast cancer, uterine cancer, colorectal cancer, melanoma, ovarian cancer, and lung cancer.[46]

**Limitations of Observational Studies**—Observational dietary studies intake are limited because the protection afforded by consumption of a particular food may be multifactorial, with several components of the food exerting potential chemopreventive effects. In addition, interpretation of observational studies can be problematic because the measured exposure may not be the true causal agent, but rather, a marker for that agent. Thus, high plasma levels of beta-carotene may be a marker for other dietary factors or lifestyle practices associated with a decreased risk of cancer.[19] Prospective, randomized trials of sufficient sample size, follow-up, and duration of treatment are needed to conclusively clarify the association between these micronutrients and cancer incidence.[18,19] Such trials are known as definitive trials, and criteria qualifying a trial as definitive have been reviewed elsewhere.[18]

**Clinical Trials**

The two large-scale cancer prevention trials discussed in this section are the Physician's Health Study and Women's Health Study, both designed with primary hypotheses for general cancer prevention. Two other highly publicized trials discussed here were designed to test primary hypotheses in specific sites—a trial by Clark et al in the skin and the ATBC Cancer Prevention Study in the lung. These latter trials are included here because, although they have site-specific primary designs, they also generated intriguing secondary findings of a broader nature that have earned them the widespread perception of having been more general prevention trials.

In a component of the Physician's Health Study, 22,071 apparently healthy, well-nourished, male physicians between the ages of 40 and 84 years were randomly assigned to supplementation with beta-carotene or placebo. After 12 years of beta-carotene supplementation, no significant early or late differences between the groups were observed with respect to the incidence of specific neoplasms or the overall incidence of malignancies. In addition, no significant differences in the incidence of cardiovascular disease or overall mortality were noted.[48]

Another ongoing study, the Women's Health Study, is a phase III primary prevention trial being conducted in Boston. In this trial, investigators are evaluating the effects of alpha-tocopherol and aspirin on the incidence of epithelial cancers, especially cancers of the lung, colon, and breast, in more than 40,000 female health professionals age 45 years or older.[19]

Although designed for skin cancer prevention, a trial of selenium, conducted by Clark et al, produced intriguing secondary analyses that have generalized its findings. This multicenter, double-blind, randomized, placebo-controlled trial in 1,312 patients with a history of basal cell or squamous cell carcinoma with a mean follow-up of 6.4 years showed that selenium treatment did not significantly protect against the development of basal cell or squamous cell carcinoma of the skin with respective risk ratios of 1.10 and 1.14. Significant reductions in total cancer mortality, total cancer incidence, and the incidences of lung, colorectal, and prostate cancers were observed, however, among patients who received oral selenium.[49]

The secondary finding regarding the effect of selenium on prostate cancer risk was a two-thirds reduction in prostate cancers (13 in the selenium group vs 35 in the placebo group).[49] Subjects in this trial had been randomly assigned to receive selenium (200 µg/d) or placebo. Follow-up time was
an average of 4½ years. The secondary finding on prostate cancer incidence has raised keen interest in selenium as a new agent for further study in patients at risk of this cancer. Other evidence supporting the effect of selenium in this setting is somewhat limited and inconsistent.[50] Small prospective US studies of selenium in persons with selenium values in the normal range hinted that a benefit could be achieved with higher selenium levels, although very small sample sizes render the results of these studies virtually noninformative.

Recently, a nested case-control study of the effects of selenium on advanced prostate cancer risk was imbedded in a prospective study design. Advantages offered by this study included: (1) the largest number of advanced prostate cancer cases (N = 181) studied to date; (2) time-integrated assessment of status through measurement of selenium levels in toenails; and (3) careful appraisal and control of other potential influencing factors.[51] In this study, the risk of advanced prostate cancer in men with the highest selenium status appeared to be one-half to two-thirds lower than that in men with the lowest selenium status. The estimated daily intake of men with the highest selenium status in this study was 159 µg/d (estimated from toenail selenium measurements in the highest selenium-level quartile).

Another agent, vitamin E (alpha-tocopherol), also has become the focus of intense interest for prostate cancer prevention, based on more intriguing secondary analyses, this time from the randomized ATBC Cancer Prevention Study involving more than 29,000 male smokers in Finland. The prostate-cancer-related secondary findings include a 40% decrease in stage B or higher prostate cancer and a 32% decrease in all stages of prostate cancer in participants receiving vitamin E. The intervention period was 5-to-8-years, and the overall number of new prostate cancer cases during this period was 99 in the vitamin E (50 mg/d) group vs 147 in the non-vitamin E group (total of 246 cases).[52] Other findings regarding vitamin E for prevention in this setting include the epidemiologic finding of a correlation between vitamin E levels and prostate cancer mortality. Androgens may increase oxidative stress of cells, which increases with age and is directly related to a variety of cancer risks. The antioxidant effects of vitamin E (as well as potential independent inhibitory effects on prostate cancer cells) may be protective in this setting. The effects of vitamin E on cellular structures (DNA and membrane), cell proteins, and immune function all suggest that this agent may reduce cancer risk. Higher prostate cancer risk was seen in association with low levels of vitamin E in a 1971-1973 study (follow-up of 17 years) of serum vitamin E levels in 2,974 subjects.[44]

Customarily in drug development, clinical trials of the most promising agents follow exploration of hypotheses through observational studies. In the cases of both selenium and alpha-tocopherol for prostate cancer chemoprevention, however, the stage is set for randomized, placebo-controlled (phase III) testing following secondary findings of two randomized, placebo-controlled clinical trials.[50] Data indicate that the activities of selenium and vitamin E are complementary and that the two agents act synergistically to inhibit carcinogenesis. Because of the high prevalence of prostate cancer and its associated morbidity and mortality, confirming either or both agents as effective chemopreventives in this setting would have a tremendous impact on public health. A phase III confirmatory trial must evaluate the effects of selenium and/or vitamin E on the primary end point of prostate cancer and on important secondary end points as well, including other cancers, total mortality, stroke, coronary heart disease, and potential chronic side effects. Strong support exists for a 2 x 2 factorial design for simultaneously testing both selenium and alpha-tocopherol.[50] Such a trial currently is being designed for multicenter implementation through the National Cancer Institute (NCI) cooperative intergroup mechanism.

**Clinical Studies in Specific Disease Sites**

**Aerodigestive Tract**

Epithelial malignancies of the aerodigestive tract (head and neck, esophagus, and lung) are the best-studied system for chemoprevention.[11,53-56] Premalignancy, second primary tumor prevention, and primary prevention trials have all been conducted in this setting.

**Surrogate End Point Biomarkers Trials**--A wide variety of surrogate end point biomarkers have been utilized in randomized trials in the aerodigestive tract. For example, the premalignant lesion, oral leukoplakia, has been used to study clinical and histologic responses to therapy because of its accessibility to biopsy and strong association with malignancy.[11,56,57]

Early studies of beta-carotene in groups at high risk of aerodigestive tract neoplasia, which used micronuclei (a marker of genetic damage) as the surrogate biomarker, found significant reductions among the group treated with beta-carotene (180 mg/wk), as compared with the placebo group.[58]
When the premalignant lesion of oral leukoplakia was used as a biomarker, however, beta-carotene alone had no significant effect on lesion remission or prevalence.[58] The combination of beta-carotene and retinol did produce a significant complete remission rate of oral leukoplakia in study participants who chewed betel nuts. In a single-arm, phase II study of vitamin E in 43 patients with oral leukoplakia, a 46% clinical response rate and a 21% histologic response rate were seen.[59] In addition, the combination of beta-carotene, retinol, and vitamin E significantly decreased the prevalence of leukoplakia when compared to placebo; also, in subjects with chronic esophagitis, the same combination produced a nonstatistically significant 34% decrease in the risk of progression or no change (as opposed to regression).[60]

In 1986, Hong et al reported that 3 months of high-dose isotretinoin had significant activity in a prospective, randomized, double-blind, placebo-controlled clinical trial of oral leukoplakia.[61] However, this high-dose, short-term approach was severely limited by prohibitive toxicity and lesion recurrence after discontinuation of therapy.

A follow-up randomized maintenance trial using low-dose isotretinoin was designed. Low-dose maintenance therapy (9 months) following a 3-month induction course of high-dose isotretinoin prevented progression significantly better than did beta-carotene.[62] Since the initial study, these patients have been followed for a median of 66 months to assess the long-term protective effect of isotretinoin. In addition, the clinical course of these subjects demonstrated a strong association between the short-term progression of oral premalignant lesions and the development of cancer. This strong correlation between long-term cancer development and short-term progression of premalignant lesions supports the validity of using oral premalignant lesion progression as a surrogate end point biomarker in head and neck cancer chemoprevention trials.[57]

The synthetic retinamides fenretinide and 4-HCR have also been evaluated in oral premalignancy. In a trial by Han et al, 4-HCR was significantly more active than placebo in reversing oral premalignant lesions in 61 patients.[63] A randomized trial evaluating the efficacy of systemic fenretinide as maintenance therapy vs no treatment after complete laser resection of oral lesions has also reported promising early findings.[64] Several lung chemoprevention trials in smokers have used progressive changes in the bronchial epithelium, such as metaplasia or dysplasia, as a study end point. An early nonrandomized trial of heavy smokers reported a substantial reduction in metaplasia in bronchoscopic biopsies, from 35% to 27%, after treatment with the synthetic retinoid etretinate.[65] Two subsequent randomized trials, however, have shown a significant spontaneous regression rate of metaplasia in the placebo arms, suggesting that the initial conclusion of retinoid activity was invalid.[66,67]

Metaplasia is thought to be a very early step in lung carcinogenesis on the basis of the substantial short-term spontaneous regression rates and rapid response to smoking cessation. Because retinoids have shown activity in the later stages of carcinogenesis, it is possible that they are active in later stages of lung premalignancy, and that lung metaplasia is not a valid marker for their efficacy. In a study from the Netherlands, beta-carotene decreased sputum micronuclei by a statistically significant 27% when compared to placebo.[68] However, beta-carotene did not reduce oxidative DNA damage, as measured by urinary excretion of 8-oxo-7,8-dihydro-2¢-deoxyguanosine, among these subjects.[2,69] A more recent trial among US male asbestos workers showed no difference in sputum atypia with retinol and beta-carotene treatment vs placebo.[70]

**Second Primary Tumor Prevention Trials**—Consistent with the concept of field carcinogenesis, the lifetime risk of second primary tumors following early-stage head and neck or lung cancer is 20% to 40%.[56,71] Hong et al conducted a 12-month, randomized, double-blind, placebo-controlled trial of high-dose isotretinoin (50 to 100mg/m²/d) as adjuvant therapy following curative therapy of primary head and neck squamous cell carcinoma.[72] Second primary tumors developed in 4% of the isotretinoin-treated patients after 32 months of follow-up, as compared with 24% of placebo-treated patients (P = .005). No significant difference was noted between the two groups with respect to primary disease recurrence (local, regional, or distant) or survival. However, one-third of the isotretinoin-treated needed dose reductions or discontinued therapy because of significant toxicities, including dry skin, cheilitis, conjunctivitis, and hypertriglyceridemia. Continued follow-up of study participants showed a protective effect of isotretinoin lasting several years after the completion of therapy.[73] Reanalysis after a median follow-up of 4.5 years showed 7 second primaries (14%) in the isotretinoin group vs 16 (31%) in the placebo group (P = .04). Analysis of second primary tumors in tobacco-exposed sites only revealed that these tumors occurred in 3 of isotretinoin-treated patients vs 13 placebo-treated patients (P = .005).
In a placebo-controlled clinical trial, Pastorino et al tested retinyl palmitate (300,000 IU/d for 12 months) in 307 patients at risk of second primary tumors following definitive therapy of primary stage I non-small-cell lung cancer. Although disease-free survival in the treatment group did not differ significantly from that in the placebo group, retinyl palmitate did significantly improve the time to development of tobacco-related second primaries.[71]

Bolla et al evaluated the use of etretinate (50 mg/d for 1 month followed by 25 mg/d for 24 months) to prevent second primary tumors following head and neck cancer. After a median follow-up of 41 months, the treatment and placebo groups had similar rates of second primaries and relapse.[74]

Two ongoing large-scale clinical trials were launched through the intergroup mechanism (an NCI-supported, national-cooperative-oncology-group mechanism) to confirm the earlier positive results of retinoids in the prevention of head and neck and lung second primary tumors. Having completed accrual in mid-1997, the lung intergroup trial involves low-dose 13cRA in nearly 1,500 patients. Final results of this trial are expected within the next 2 years, after treatment and follow-up of the last patients.[75] The head and neck second primary tumor intergroup trial also is testing low-dose 13cRA and is expected to complete accrual by the end of 1998.[76-78]

In 1988, the multicenter Euroscan trial was begun in patients previously treated for lung or head and neck cancer. This study is using a 2 × 2 factorial design to study the efficacy of retinyl palmitate and N-acetylcysteine in preventing second primary tumors.[79]

**Primary Chemoprevention**—Cancer incidence and mortality have also been used as study end points in randomized trials of individuals at risk of neoplasia on the basis of nutrient deficiencies, premalignant histology, or high-risk exposures. Two nutrition intervention trials were conducted in Linxian, China. In the first of these trials, 29,584 residents in four communities with documented low dietary intake of several micronutrients, including beta-carotene, and high rates of esophageal and gastric cancer mortality, were randomly assigned to 5 years of therapy with one of four potential combinations: (1) retinol plus zinc, (2) riboflavin plus niacin, (3) vitamin C and molybdenum, (4) and beta-carotene, vitamin E, and selenium.

Subjects receiving the combination of beta-carotene, vitamin E, and selenium had 13% lower total cancer mortality than those not receiving the combination (relative risk [RR], 0.87; 95% confidence interval [CI], 0.75 to 1.00); this difference did not reach statistical significance. The combination group also had a 21% decrease in gastric cancer deaths (RR, 0.79; 95% CI, 0.64 to 0.99), which was marginally statistically significant.[80] Because nutrients were studied in combination, individual effects could not be distinguished.

The other Linxian trial randomized residents with esophageal dysplasia to placebo or a multivitamin/multimineral combination that included beta-carotene, alpha-tocopherol, and selenium.[81] Patients receiving the multivitamin/multimineral combination showed no statistically significant changes in esophageal, stomach, or total cancer mortality.

The ATBC Cancer Prevention Study randomized 29,133 Finnish male smokers (50 to 69 years old), in a 2 × 2 factorial design, to 5 to 8 years of treatment with beta-carotene, vitamin E, beta-carotene plus vitamin E, or placebo.[82] No protective effect against lung cancer was noted for either treatment. In fact, the beta-carotene group had a statistically significant, 18% higher risk of lung cancer.

The findings of another multicenter, randomized, double-blind, placebo-controlled primary prevention trial, CARET, supported the results of the ATBC study.[83,84] In CARET, 18,314 smokers, former smokers, or workers exposed to asbestos were treated with a combination of beta-carotene plus retinol or placebo. On the basis of increased risks of lung cancer incidence and mortality and the evidence from the ATBC study, CARET was stopped 21 months earlier than planned. Compared to the placebo group, the active-treatment group had a relative risk of lung cancer incidence of 1.36 (95% CI, 1.07 to 1.73; P = .01) and a relative risk of lung cancer mortality of 1.59 (95% CI, 1.13 to 2.23; P = .01).

The increase in lung cancer following supplementation with beta-carotene and retinol was seen among current but not former smokers. Additional subgroup analyses also suggested associations of excess lung cancer incidence with the highest quartile of alcohol intake (RR, 1.99; 95% CI, 1.28 to 3.09; P = .01) and with large cell histology (RR, 1.89; 95% CI, 1.09 to 3.26; P = .35).[2,84]

**Skin**

The efficacy of the chemopreventive agents, isotretinoin, beta-carotene, and selenium has been assessed in patients at risk of developing non-melanoma skin cancer. High-dose isotretinoin was found to prevent new skin cancer in individuals with xeroderma pigmentosum. After 2 years of treatment, subjects had an average 63% reduction in skin cancers. After drug discontinuation, the tumor frequency increased a mean 8.5-fold over the frequency during treatment. Patients treated
with isotretinoin were also noted to experience mucocutaneous, hepatic, and skeletal toxicities, as well as hypertriglyceridemia.[85]
In contrast, phase III trials using lower net doses in lower-risk individuals have reported mixed results. In a randomized clinical trial of patients previously treated for basal cell carcinoma, 36 months of treatment with low-dose isotretinoin failed to produce a statistically significant difference in the occurrence of new basal cell carcinomas. In addition, the treated group developed side effects associated with isotretinoin, including elevated serum triglycerides, hyperostotic axial skeletal changes, and mucocutaneous reactions.[86]

Two additional chemoprevention trials have been conducted to evaluate the effect of retinoids on two primary end points: squamous cell carcinoma and basal cell carcinoma. The Skin Cancer Prevention-Actinic Keratoses (SKICAP-AK) trial randomly assigned over 2,000 moderate-risk patients with a history of more than 10 actinic keratoses and at most two prior skin cancers to 5 years of treatment with retinol (25,000 IU/d) or placebo. Compared with the placebo recipients, the subjects supplemented with retinol had a significant reduction in squamous cell carcinoma incidence (P = .04) but no reduction in basal cell carcinomas.[87]

The Skin Cancer Prevention-Squamous Cell Skin Cancer and Basal Cell Skin Cancer (SKICAP-S/B) trial randomly assigned high-risk individuals with a history of four or more skin cancers to one of three arms: 25,000 IU/d of retinol, 5 or 10 mg/d of isotretinoin, or placebo, all for 3 years. No significant benefits of retinoid treatment on the prevention of squamous cell cancer or basal cell skin cancer were observed in this high-risk group.[88]

Other agents have been evaluated as potential chemopreventives for skin cancer. In one trial, over 1,800 patients with a recently resected nonmelanoma skin cancer were randomized to beta-carotene or placebo for 5 years. There was no significant difference between the beta-carotene and control groups with respect to the rate of occurrence of the first new nonmelanoma skin cancer or the mean number of new nonmelanoma skin cancers per patient-year. In addition, subgroup analyses showed no efficacy of active treatment, either in patients whose initial plasma beta-carotene level was in the lowest quartile or in current smokers.[89]

A multicenter, double-blind, randomized, placebo-controlled trial in 1,312 patients with a history of basal cell or squamous cell carcinoma with a mean follow-up of 6.4 years showed treatment with 200 µg of oral selenium in a brewer’s yeast tablet did not significantly protect against the development of basal cell or squamous cell carcinoma of the skin.[49] However, as discussed in detail above, significant reductions in total cancer mortality, total cancer incidence, and incidences of lung, colorectal, and prostate cancers were observed among patients who received selenium.[50,90]

**Cervix**

The cervix is well-suited to chemoprevention trials because of its accessibility to clinical and histologic monitoring. In addition, the histologic progression of early atypia through intraepithelial lesions to cancer is a well-studied surrogate biomarker for trials.[91] Cervical intraepithelial neoplasia (previously referred to as dysplasia) is graded 1, 2, or 3 along a spectrum of increasing neoplastic changes and risk of progression to invasive cancer.

The Bethesda System, used for cytology but not yet for histologic specimens, considers CIN I to be low-grade squamous intraepithelial lesions (LSILs), and CIN 2 and 3 to be high-grade squamous intraepithelial lesions (HSILs). The distinction between LSILs and HSILs reflects the biological processes that underlie the histologic patterns and, thus, highlights important differences in their potential to progress to invasive cancer. Notably, the risk of progression of LSILs, which includes condylomatous changes, koilocytic atypia, and CIN I, is significantly lower than that of HSILs, which encompass CIN 2 and CIN 3.[91,92]

Multiple case-control studies have found that high dietary intake of carotene and possibly of vitamins C and E and folate is associated with a reduced risk for cervical cancer.[25,39] Epidemiologic studies of patients with LSIL, HSIL, or cervical cancer suggest that low intake of beta-carotene is also associated with increased risk. Case-control and prospective blood-based studies demonstrate an inverse correlation between beta-carotene and the presence of intraepithelial lesions.[25,39]

Folate deficiency is hypothesized to play a role in cervical carcinogenesis by facilitating the integration of HPV at a fragile genomic site. In a review of the literature, Potischman noted that, in contrast to other protective micronutrients, folate deficiency correlated most closely with the earliest stages of dysplasia and not with high-grade lesions or invasive cancer.[39]

De Vet et al compared beta-carotene to placebo in patients with CIN and found no significant differences in regression rates.[93] Meyskens and Manetta completed a phase II trial of beta-carotene in 30 patients with biopsy-proven CIN 1 and CIN 2. After treatment with 30 mg/d of beta-carotene for up to 6 months, more than 70% of the patients responded, as assessed by a
negative colposcopy and Pap smear.[94]
In a randomized trial, 235 patients with CIN I and 2 were treated with 10 mg of folate or with 10 mg of vitamin C (used as a placebo) for 90 days. After 6 months of follow-up, the rate of lesion regression did not differ significantly between the two groups. The investigators felt that the minimal dose of vitamin C was unlikely to have therapeutic effects and, thus, was an acceptable placebo. In support of this, they demonstrated no difference between serum vitamin C levels of subjects and controls.[95]
A Southwest Oncology Group multicenter, randomized, double-blind, placebo controlled trial of folate in individuals with koilocytic atypia, CIN 1, or CIN 2 also had negative results.[96] Because all of the patients in these two randomized studies had established squamous intraepithelial lesions, however, neither trial addressed the possibility that folate supplementation prior to the development of cervical lesions may prevent the occurrence of neoplasia. In addition, studies involving patients with HSILs (CIN 2 and CIN 3), which have a significantly higher rate of progression to invasive cancer, may be more informative.[91]
Although most nutritional studies do not correlate decreased vitamin A levels with squamous intraepithelial lesions, a considerable amount of experimental data suggests that vitamin A and its derivatives inhibit HPV-associated proliferation. In a phase I/II trial of topical retinyl acetate gel in patients with CIN 1 and 2, Romney et al showed that high compliance could be achieved and determined the dose for a phase III study.[97] Phase I trials by Surwit et al, Meyskens et al, and Weiner et al, demonstrated the safety of topical application of ATRA to the cervix and showed that a dose of 0.372 mg/dL or higher was more likely to achieve a response.[98-100]
Meyskens et al reported the results of a randomized phase IIb trial utilizing the same dose of beta-all-trans-retinoic acid in 151 patients with CIN 2 and 150 patients with CIN 3.[101] Participants were randomized to treatment with cervical caps containing the retinoid or placebo. The complete histologic regression rate of CIN 2 was 27% in the placebo group and 43% in the treatment group (one-sided P = .041). No difference in regression rate was noted between the CIN 3 groups. Although vulvar and vaginal side effects occurred in the treatment group, they were mild, reversible, and seen in less than 5% of patients. Major difficulties with patient compliance, however, and the loss of 52 patients to follow-up limit the interpretation of this trial.[2,101]
Additional promising agents, such as 4-HPR, and combinations of retinoic acid derivatives and interferon, are currently being studied in phase I and II trials. In addition, validation and testing of potential surrogate end point biomarkers continue in these and other trials.[91,102]
**Breast**
Observational studies provide some evidence that breast cancer risk is reduced by increased dietary retinoids.[41] Retinoids have been shown to have breast cancer-inhibitory effects through the up-regulation of TGF-beta, as well as through differentiation.[1,16] In addition, investigators are exploring the effects of fenretinide on insulinlike growth factor-1 (IGF-1), which is thought to play a role in breast tumorigenesis.[103]
The Fenretinide (4-HPR) Breast Cancer Study is a randomized, multicenter clinical trial designed to evaluate the effectiveness of 5 years of fenretinide (200 mg/d) in reducing the incidence of contralateral breast cancer in patients who had undergone prior surgery for breast cancer. Between 1987 and 1993, 2,972 women between the ages of 30 and 70 years were enrolled. Although final results of this trial are not yet published the investigators have reported an inhibitory effect of fenretinide on the occurrence of second primary cancers among premenopausal women.[1,2,11,18,36] There are three large-scale trials of tamoxifen in women at varying degrees of risk of breast cancer, including the recently completed Breast Cancer Prevention Trial (P-1), which will be discussed in part 2 of this review.
**Colon**
Prospective dietary studies have shown an inverse association between vitamin intake and colon cancer.[11,104] However, randomized, controlled clinical trials have not confirmed these findings.[11,105,106]
Beta-carotene has been studied alone or in combination with other agents in patients with a history of resected adenomas in three randomized trials. In one trial involving 291 patients, beta-carotene (15 mg/d) did not alter the rate of polyp recurrence compared to placebo.[2]
In another placebo-controlled trial utilizing a 2 × 2 factorial design, 864 patients were randomized to beta-carotene and/or a vitamin C and E combination. Rates of adenoma recurrence did not differ significantly among any of the groups.[105]
Also using a placebo-controlled, factorial design, the Australian Polyp Prevention Project randomized patients to combinations of beta-carotene, a wheat bran diet, and/or a reduced fat diet (25% calories
from fat), for a total of seven intervention groups and one control group. No statistically significant differences in recurrence of adenomas were seen among any of the groups. The most promising approaches for colon cancer prevention involve NSAIDs and calcium, which will be discussed in Part 2 of this review.

**Bladder**

In vivo animal model, in vitro, and epidemiologic studies have shown that retinoids are active against bladder carcinogenesis. Although initial cure rates of superficial bladder cancer are high, in a series of patients who were followed for a minimum of 20 years or until death, the risk of bladder cancer recurrences was over 80% in certain subgroups. Data show that intravesical bacillus Calmette-Guerin (BCG) immunotherapy reduces long-term tumor recurrence, disease progression, and mortality. However, the use of BCG reduces tumor recurrence by an average of only 40%. Two factors make neoplastic lesions of the bladder an ideal site for evaluating chemopreventive agents: (1) the accessibility of the lesions to observation and tissue acquisition; and (2) the significant risk of recurrence in a population successfully treated for a primary lesion. Three randomized clinical trials evaluated etretinate in patients following resection of their superficial bladder tumors. Although the three studies encountered substantial mucocutaneous toxicity, prolonged, low-dose etretinate was effective in two trials. These studies were limited by small patient numbers and short-term follow-up, however. The synthetic retinoid fenretinide has a more favorable toxicity profile than etretinate and has shown promising results in a phase IIa study. In addition, in a double-blind, randomized trial, megadose vitamins A, B6, C, and E plus zinc decreased bladder tumor recurrence among patients receiving BCG immunotherapy. The control group, which received the recommended daily allowance (RDA) of the vitamins, had a 80% recurrence rate (24 of 30 patients). This was significantly higher than the 40% recurrence rate (14 of 35 patients) seen in the high-dose group (P = .001). Additional studies of the individual micronutrients in larger patient groups and longer follow-up will help determine the role of chemoprevention in bladder cancer.

**Liver**

An acyclic retinoid, polyprenoic acid, has been shown to inhibit hepatocarcinogenesis and to induce differentiation and apoptosis in laboratory models. In a randomized, placebo-controlled clinical trial, 89 patients with hepatocellular carcinoma (hepatoma) who were free of disease after primary therapy were randomized to 600 mg of oral polyprenoic acid or placebo daily for 12 months. After a median follow-up of 38 months, 12 patients (27%) in the polyprenoic acid group had recurrent or new hepatomas, as compared with 20 patients (49%) in the placebo group (P = .04). Polyprenoic acid reduced the occurrence of second primary hepatomas, showing a statistically significant adjusted relative risk of 0.35 (95% CI, 0.12 to 0.78).

**Summary**

This concludes our review of retinoids and carotenoids and other classic antioxidants. Part 2 of this article, which will appear in next month’s issue, will focus on several other groups of chemopreventive agents. These include hormonally mediated chemopreventive agents, such as tamoxifen, finasteride, oral contraceptives, and DHEA; nonclassic antioxidant natural agents, such as calcium, the polyphenols, the isothiocyanates, and genistein; NSAIDs, such as sulindac sulfone, aspirin, and celecoxib; DFMO; oltipraz; and N-acetylcysteine.

**References:**


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