COMMENTARY
Cancer chemoprevention: principles and prospects

Mark A. Morse and Gary D. Stoner
Laboratory of Cancer Chemoprevention and Etiology, Department of Preventive Medicine, The Ohio State University, CHRI Suite 1148, 300 West Tenth Avenue, Columbus, OH 43210, USA

Introduction
Cancer chemoprevention can be defined as prevention of cancer by the administration of one or more chemical entities, either as individual drugs or as naturally occurring constituents of the diet. For several reasons, chemoprevention has received growing consideration as a means of cancer control. In the USA, the 5 year survival rate of cancer patients is currently 51% overall (1). Among those organ sites with exceptionally low 5 year survival rates are lung (13%), ovary (39%), stomach (16%), esophagus (9%) and pancreas (3%) (1). Clearly, the considerable advances that have occurred in earlier detection and treatment of cancer have done little to improve the prognosis for patients diagnosed with cancer at certain organ sites. Thus, the aggressive prevention of cancer should be a cornerstone of any cancer control policy. Primary cancer prevention requires removal of exposure of individuals to etiologic agents. However, numerous subpopulations at high risk for certain types of cancer may already have received considerable exposure to such agents, and many human cancers cannot be ascribed to specific etiologic agents. Thus, preventative strategies that do not require prior knowledge of specific etiologic factors have great appeal. Additionally, the cost of chemopreventative programs in a given population should be considerably less than the actual health care costs incurred by any fraction of that population that develops cancer. Hence, chemoprevention of cancer may become a major weapon in the anticancer arsenal.

Target populations
Presently, the projected target populations for cancer chemoprevention are comprised of high-risk groups, such as: (i) individuals that engage in risk-taking behaviors or lifestyles (e.g. smokers and users of snuff); (ii) individuals that have received occupational exposure to known carcinogens (e.g. asbestos workers); (iii) those that are known to be genetically predisposed to the development of cancer (e.g. individuals with familial colonic polyposis, etc.); (iv) individuals that possess premalignant lesions (e.g. oral leukoplakia in snuff users); (v) survivors of primary cancers with a high degree of recurrence or a high tendency towards formation of second primary tumors; and (vi) cancer survivors that received chemotherapy and/or radiation therapy. Some controversy remains as to whether or not chemopreventative strategies (other than certain dietary measures) will or should be used in the general population.

Ideal qualities of chemopreventative agents
The ideal chemopreventative agent should have the following qualities: (i) little or no untoward or toxic effects; (ii) high efficacy; (iii) capability of oral administration; (iv) a known mechanism of action; and (v) low cost. With regard to the first requirement, it is obvious that such agents should have as few untoward effects as possible. In any chemopreventative dosing regimen, healthy individuals will be administered a drug chronically, possibly for life. Only certain high-risk populations (e.g. survivors of a primary tumor) can be reasonably expected to endure mild toxicity or discomfort in the use of a chemopreventative agent. The efficacy should be rigorously demonstrated, first by success in in vivo animal models, and then in clinical trials. In no case should the potential of a chemopreventative agent be judged by epidemiologic data alone. The requirement of an oral dosage form allows self-medication and enhances subject compliance. The primary exceptions to this requirement are topical preparations used for the prevention of skin cancer. Knowledge of the precise mechanism(s) of action of the prospective chemopreventative agent will decrease the possibility of untoward interactions with other administered drugs or dietary constituents. Finally, it is understood that the cost of a given chemopreventative regimen must be low, since the agent(s) will be chronically administered.

Classes of chemopreventative agents
Absolute classification of all known chemopreventative compounds is difficult due to the fact that the precise mechanism(s) of action are not known for many compounds. The classification scheme developed by Wattenberg (2) is based essentially upon the time period that agents appear to have activity in animals models of carcinogenesis. On this basis, the three major types of chemopreventative agents are: (i) inhibitors of carcinogen formation; (ii) 'blocking' agents; and (iii) 'suppressing' agents (2). Blocking agents are inhibitors of tumor initiation, while suppressing agents can be described more specifically as inhibitors of tumor promotion/progression. We have further differentiated both blocking agents and suppressing agents, based on mechanistic principles (Tables I and II). It should be noted that many well-characterized compounds exhibit several discrete mechanisms of action. Thus, in the examples below, many of the compounds belong to more than one class.

Inhibitors of carcinogen formation
The majority of compounds that inhibit the formation of carcinogens act to prevent the formation of nitrosamines from secondary amines and nitrite in an acidic environment. When present in appreciable amounts, ascorbic acid can decrease nitrosamine production (3). Other compounds that inhibit nitrosamine formation include phenols such as caffeic acid and ferulic acid (4) as well as several different sulfhydryl compounds

*Abbreviations: GST, glutathione S-transferase; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-l-butaneone; NMBA, N-nitrosomethylbenzylamine; DC, indole-3-carbinol; BPDE, benzo[a]pyrene diol epoxide; ODC, ornithine decarboxylase; TPA, 12-O-tetradecanoylphorbol-13-acetate; DFMO, a-difluromethylornithine; PKC, protein kinase C; EGCG, epigallocatechin gallate; PCNA, proliferating cell nuclear antigen. BrdU, bromodeoxyuridine.

© Oxford University Press

Carcinogenesis vol 14 no 9 pp 1737–1746, 1993
Table I. Categories of blocking (anti-initiating) agents

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhibition of cytochrome P450</td>
<td>dithiocarbamates, isothiocyanates, diallyl sulfide, ellagic acid</td>
</tr>
<tr>
<td>Induction of cytochrome P450</td>
<td>indole-3-carbinol, β-napthoflavone</td>
</tr>
<tr>
<td>Induction of phase II enzymes</td>
<td>isothiocyanates, polyphenols, dihydrothiols</td>
</tr>
<tr>
<td>Scavenging electrophiles</td>
<td>ellagic acid, N-acetylcysteine, sodium thiosulfate</td>
</tr>
<tr>
<td>Induction of DNA repair</td>
<td>vanillin</td>
</tr>
</tbody>
</table>

Table II. Categories of suppressing (antipromotion/antiprogession) agents

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhibition of polyamine metabolism</td>
<td>α-difluoromethyl-ornithine, substituted putrescines</td>
</tr>
<tr>
<td>Inhibition of arachidonic acid metabolism</td>
<td>piroxicam, indomethacin, aspirin, quercetin, curcumin</td>
</tr>
<tr>
<td>Protease inhibition</td>
<td>tosyl phenylalanine, chloromethyl ketone, antipain, Bowman-Birk protease inhibitor</td>
</tr>
<tr>
<td>Induction of differentiation</td>
<td>retinoids, calcium, vitamin D</td>
</tr>
<tr>
<td>Inhibition of oncogene expression</td>
<td>lovastatin, limonene, antisense, oligonucleotides</td>
</tr>
<tr>
<td>Inhibition of product post-</td>
<td>lovastatin, limonene, antisense, oligonucleotides</td>
</tr>
<tr>
<td>translational modification</td>
<td></td>
</tr>
<tr>
<td>Inhibition of transcription</td>
<td>staurosporine, three-di-hydrospingsolene</td>
</tr>
<tr>
<td>or translation</td>
<td></td>
</tr>
<tr>
<td>Inhibition of protein kinase C</td>
<td>sarkophyll A, epigallocatechin gallate, selenium</td>
</tr>
<tr>
<td>Inhibition of oxidative DNA damage</td>
<td></td>
</tr>
</tbody>
</table>

Blocking agents

There are several means of chemical intervention in the initiation stage. Except for direct-acting carcinogens, genotoxic carcinogens must first be metabolically activated to electrophilic forms that can damage DNA, while to some extent, avoiding pathways of metabolic detoxification. The electrophilic species must then react with DNA, ideally forming adducts that result in mispairing and are poorly repaired, thus resulting in mutation. On this basis, the vast majority of blocking agents can be assigned to one or more of five major categories, namely (i) inhibitors of cytochrome P450 enzymes; (ii) inducers of cytochrome P450 enzymes; (iii) inducers of phase II enzymes, such as glutathione S-transferase (GST*); (iv) nucleophilic compounds that can act as scavengers of electrophiles; and (v) inducers of DNA repair (Table I).

One of the first cytochrome P450 inhibitors shown to have chemopreventative activity was disulfiram, which inhibits the activation of dimethylhydrazine (7) and colon neoplasia induced by this compound (8). Diallyl sulfide, a naturally occurring constituent of Allium vegetables, inhibits carcinogen activation (9) and tumorigenesis in a number of animal models (10-13). The isothiocyanates are among the most potent chemopreventative agents known (14-22). For example, dietary phenethyl isothiocyanate at a concentration of 3 μmol/g diet can inhibit 4(−methylthiuronium)-1-(3-pyridyl)-1-butanone (NNK*)−induced lung tumors in F344 rats by ~50% (17) and completely inhibit N-nitrosomethylbenzylamine (N MBA)-induced esophageal tumors in F344 rats (21); also, 6-phenylhexyl isothiocyanate can inhibit NNK-induced lung tumorigenicity in strain A mice by >80% when administered at a dose of 50-fold lower than NNK (22). Ellagic acid inhibits NMBA metabolism in vivo and in vitro (23,24) and inhibits NMBA-induced esophageal tumors (25,26).

Inducers of cytochrome P450 can act as blocking agents as well, by increasing the production of activated metabolites at resistant non-target tissues or by enhancing oxidative detoxification at any tissue site. Indole-3-carbinol (I3C) is a potent inducer of cytochrome P450 enzymes and has chemopreventative activity in a number of animal models (27-33). In at least some of these models, the inductive abilities of I3C directly account for the observed inhibition of tumorigenicity. However, the induction of P450 activity by I3C can lead to a shift in target organ through enhanced activation of carcinogen elsewhere (33,34); this may, at least in part, account for the known cocarcinogenic or promotional activity of I3C (35,36).

Specific inducers of phase II enzymes are preferred to cytochrome P450 inducers as chemopreventative agents, since they inhibit a greater range of target carcinogens and are less likely to produce cancers themselves. Benzyl isothiocyanate (37), phenethyl isothiocyanate (38) and sulforaphane (39) are examples of isothiocyanates that induce GST; as previously stated, many isothiocyanates are also excellent inhibitors of carcinogen activation. The dithiolethione, oltipraz, is a potent inducer of GST and inhibits carcinogen-induced tumorigenesis in a number of animal models (40-46).

Trapping agents or scavenging agents are compounds that physically react with the activated (electrophilic) forms of carcinogens. Potential scavenging agents include such compounds as ellagic acid, N-acetylcysteine and sodium thiosulfate. Ellagic acid reacts directly with the diolepoxide of benzo[a]pyrene (BPDE) to form a conjugate (47); such activity may account for its inhibition of BPDE-induced mutagenicity and carcinogenicity (48,49). The sulfhydryl moiety of N-acetylcysteine can accept electrophilic species, which may account for its antimutagenic and anticarcinogenic effects (50-52). Sodium thiosulfate reacts
with β-propiolactone and inhibits its mutagenicity and carcinogeticity (53). A major disadvantage of scavenging agents is that they must be maintained at discernible concentrations in target tissues at all times during which carcinogens are present.

Alterations in DNA repair have long been known to affect mutagenic or tumorigenic outcomes in prokaryotic and eukaryotic systems. An example of a naturally occurring chemical that affects DNA repair is vanillin, which inhibits bacterial mutagenicity (54–56), chromosomal aberrations in Drosophila melanogaster (57) and mammalian cell mutagenicity (58–62). Of considerable potential as a chemopreventative strategy would be the induction of O^alkylguanine-DNA-alkyltransferase, the enzyme that repairs O^alkylguanine lesions. Unfortunately, only highly toxic and/or carcinogenic conditions are known to result in induction of this enzyme (63–66).

Many would argue that the use of blocking agents is not feasible in the human population, since all members of high-risk groups have presumably received some exposure to etiologic (initiating) agents. The work of Vogelstein et al. (67,68) on colorectal cancer suggests that human cancer is not adequately represented by the traditional initiation—promotion model, but more likely involves an accumulation of several different genetic events. If so, then administration of blocking agents should prove of some value, since many individuals at high risk (e.g. smokers and the occupationally exposed) are continually exposed to genotoxic carcinogens. Individuals that are genetically predisposed to cancer development must avoid further mutations that could trigger the carcinogenic process; such individuals will be excellent candidates for prophylactic treatment with blocking agents starting as early in their lifetimes as is practical. Also, the administration of inhibitors of promotion/progression will be helpful in combatting the effects of exposure to a wide range of carcinogens, no matter what model human carcinogenesis follows. Ultimately, the best strategy for cancer chemoprevention may be the administration of combinations of blocking and suppressing agents.

Suppressing agents

Classification of suppressing agents is considerably more difficult since the critical events and the precise sequence of events in the processes of promotion and progression are poorly understood. However, many current suppressing agents can be categorized as: (i) inhibitors of polyamine metabolism; (ii) inhibitors of arachidonic acid metabolism; (iii) protease inhibitors; (iv) inducers of differentiation; (v) inhibitors of oncogene expression; (vi) inhibitors of protein kinase C; and (vii) inhibitors of oxidative DNA damage. Many other mechanistic approaches for inhibition of tumor promotion/progression are currently in development (69).

The polyamine content of cells is correlated to their proliferative, and often, their neoplastic capabilities (70). A key enzyme in the polyamine biosynthetic pathway, ornithine decarboxylase (ODC), catalyzes the conversion of ornithine to putrescine (71). Phorbol ester promoters such as 12-O-tetradecanoylphorbol-13-acetate (TPA) cause increased ODC activity and accumulation of polyamines in affected tissues (72). Inhibitors of polyamine metabolism are exemplified by a suicide inhibitor of ODC, α-difluoromethylornithine (DFMO) (73). Not surprisingly, DFMO inhibits tumorigenesis induced by a number of different carcinogens (74–81). Because of the rapid turnover of ODC (70), constant levels of a given ODC inhibitor must be maintained at the desired target organ to achieve the desired antiproliferative activity.

Among the myriad changes that occur during experimentally induced tumor promotion is an increased metabolism of arachidonic acid, which contributes to an overall inflammatory response (70). The cyclo-oxygenase pathway converts arachidonic acid to prostaglandins, prostacyclins and thromboxanes, while lipoxygenase converts arachidonic acid to 12-hydroperoxyarachidonic acid and 12-hydroxyarachidonic acid (82). The cyclo-oxygenase inhibitors piroxicam and indomethacin have been found to inhibit experimental colon cancer (80,83–85), while ibuprofen has activity against bladder tumorigenesis (86). The lipoxygenase inhibitors 3,4,2',4'-tetrahydroxychalcone and quercetin inhibited ODC induction and the promotion of 7-bromomethylbenz[a]anthracene-initiated epidermal tumors (87). Curcumin, the major pigment in mustard and turmeric, effectively inhibits cyclo-oxygenase and lipoxygenase activities (88) as well as TPA-induced promotion in mouse skin (89).

Protease inhibitors are active inhibitors of the early stages of promotion in mouse skin models (90), and are also effective in the suppression of bladder, breast, colon, liver and lung tumorigenesis (91–94). They have been shown to prevent transformation of NIH 3T3 cells by the H-ras oncogene (95), and inhibit the formation of promoter-induced oxygen radicals and hydrogen peroxide (96). Protease inhibitors that can inhibit chymotrypsin are more potent in the inhibition of oxygen radicals, and appear to be better inhibitors of tumor promotion (97). Untoward effects of protease inhibitors include pancreatic enlargement in some experimental species and decreased rates of growth in young animals (98).

Retinoids are known to induce the differentiation of epithelial cells in vivo and in vitro (99,100). It is known that retinoids bind to nuclear receptors and can regulate the expression of certain genes (100). The effects of retinoids on cell growth and on in vivo transformation are well correlated with their abilities to affect gap junctional communication (101–103). Retinoids are effective in inhibiting tumorigenesis in a number of animal model systems (104–113), and are the focus of many clinical trials (114,115). Among the potential problems with the use of retinoids is their tendency to induce hepatotoxicity and teratogenicity in animals (114); also, as might be expected for vitamin A analogs, tumors appear to have a growth requirement for retinoids (116,117). Calcium and vitamin D, like retinoids, stimulate cell differentiation (118) and inhibit tumor promotion in animal models, particularly when high fat diets are employed (119–121).

Other potential targets for chemoprevention include the post-translational modification of oncogene products. For example, ras proto-oncogene activation has been found in a number of human and rodent tumors (122–128). Additionally, ras activation seems to be an event that occurs relatively early in the carcinogenic process (129–131). The protein product of the ras gene, p21, undergoes farnesylation (132), which appears to be critical for its location in the plasma membrane and its ability to transform cells (133). d-Limonene apparently inhibits p21 farnesylation and is an effective chemopreventative agent (134–142). This is most likely due to the inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase (143). A more specific means of interference with ras farnesylation would be inhibition of the recently purified ras farnesyl:protein transferase (144).

Antisense oligonucleotides targeted against specific oncogenes represent still another proposed approach in the chemoprevention of cancer. ‘Normal’ phosphodiester oligonucleotides would not be of much use, because of their limited stability; currently the best characterized oligonucleotide agonists are phosphorothioates.
A primary mode of action of such compounds could be through transcriptional arrest via interaction with double-stranded DNA (145). Other possible mechanisms include translational inhibition (145) and inhibition of peptide chain elongation (146). The ultimate appeal of such compounds may be their specificity; one can envisage a specially constructed antisense oligonucleotide that would affect an activated oncogene only, and not its proto-oncogene precursor. However, there are potential problems associated with such compounds. First of all, these drugs cannot be given orally. Also, true disruption of gene expression will require constant exposure to the drug.

Another possible target for chemopreventative intervention is protein kinase C (PKC). PKC actually constitutes a family of several isozymes that are activated by diacylglycerol (147,148). The potent tumor promoter, TPA, binds to, and activates PKC, in competition with diacylglycerol (149). PKC stimulation results in phosphorylation of regulatory proteins that affect cell proliferation. Known chemopreventative agents that have some inhibitory activity towards PKC include glycyrrhetic acid and tamoxifen (150); of course, much of the inhibitory effects of these compounds is due to other mechanisms of action. Staurosporine, an inhibitor of the catalytic site of PKC and threo-dihydrosphingosine, an inhibitor of the regulatory site of PKC both have potential as chemotherapeutic agents (151,152); these compounds have not been tested for chemopreventative activity.

Oxidative DNA damage is induced by a number of promoters and complete carcinogens, and appears to be of critical importance in stage I of promotion and in tumor progression; the production of superoxide, hydroxyl radicals and hydrogen peroxide can result in the formation of many DNA adducts, including 8-hydroxydeoxyguanosine, thymine glycol and 5-hydroxymethyl uracil (153). Indeed, the formation of such DNA adducts from reactive oxygen species after treatment with promoters has forced a re-evaluation of the use of the term 'genotoxic'. SarcophytoI A, a diterpene isolated from soft coral, inhibits TPA-induced phagocytic infiltration, formation of oxidative species and oxidation of DNA bases (154) as well as inhibiting tumor promotion (155). Additionally, epigallocatechin gallate (EGCG), a constituent of green tea, decreases TPAs-promoters has forced a re-evaluation of the use of the term 'genotoxic'. Sarcophytol A, a diterpene isolated from soft coral, inhibits TPA-induced phagocytic infiltration, formation of oxidative species and oxidation of DNA bases (154) as well as inhibiting tumor promotion (155). Additionally, epigallocatechin gallate (EGCG), a constituent of green tea, decreases TPA-induced increases in H2O2 and oxidized DNA bases (153).

Under conditions in which EGCG did not affect NNK-induced DNA methylation, EGCG reduced both 8-hydroxydeoxyguanosine formation and tumorogenesis in lung induced by NNK (156). Inhibition of oxidative DNA damage may well be the mechanism by which a number of other compounds with antioxidant activity inhibit carcinogenesis.

Unlike the past (and present), where agents were selected by serendipity or more recently, by massive screening systems, future development of potential chemopreventative agents will probably proceed via intentional design. Whilst it is hoped that newly designed drugs with the above mechanisms will be somewhat more specific than currently available compounds, it is understood that all of the above processes are required by normal cells, preneoplastic cells and neoplastic cells, and that ultimate gains in selectivity and decreased toxicity may be limited.

Use of intermediate biomarkers in chemoprevention studies

Biomarkers may serve as a means to detect exposure to carcinogenic influences, as prognostic or diagnostic indicators, or as intermediate endpoints in intervention trials. The economic burden of chemopreventative clinical trials requires cost-effective means of achieving results. In the USA, clinical trials of drugs are conducted in three phases. Traditionally, phase I clinical trials are conducted in normal individuals with the goals of determining the pharmacokinetics, metabolism and toxicity of the test compounds. Phase II clinical trials are tests of efficacy conducted on a small scale in persons with the relevant disease state while phase III trials are long-term large-scale tests of efficacy. A difficulty with chemoprevention trials is that the subjects used are presumed to be cancer free. Since cancer may still be a relatively rare occurrence, even in high-risk groups, the use of cancer as an endpoint would require design of large and lengthy phase II and III trials, adding many years and great cost to a process that is already slow, complicated and expensive. As a result, the use of biomarkers as intermediate endpoints in chemoprevention trials has become a necessity for the efficient examination of prospective chemopreventative agents.

Kelloff et al. (157) classified intermediate endpoints as genomic biomarkers, markers of cell proliferation, markers of cell differentiation and premalignant lesions (Table III). Examples of genomic biomarkers include markers of oncogene activation, markers of tumor suppressor gene inactivation, alterations in DNA methylation patterns, alterations in cellular DNA content and carcinogen—DNA adducts. Sufficiently sensitive techniques now exist to allow the detection of oncogene activation in animal models a few weeks after carcinogen administration, long before actual tumors form (129). The ras family of proto-oncogenes, mutated to active forms in many human cancers and usually at an early stage, would be a prime candidate as an intermediate biomarker. For example, K-ras activation, an early change in mouse lung tumorogenesis, occurs in a high proportion of human lung adenocarcinomas (158). In one report, mutations in K-ras were found in DNA isolated from the stool of patients with adenomas or adenocarcinomas of the colon (159). Mutations in the p53 gene are among the most frequently observed genetic changes in tumor suppressor genes in human cancers (160—163).

Hypomethylation of genomic DNA is associated with increased

### Table III. Intermediate biomarkers

<table>
<thead>
<tr>
<th>Class</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genomic biomarkers</td>
<td>oncogene activation</td>
</tr>
<tr>
<td></td>
<td>tumor suppressor gene inactivation</td>
</tr>
<tr>
<td></td>
<td>altered DNA methylation</td>
</tr>
<tr>
<td></td>
<td>abnormal DNA content</td>
</tr>
<tr>
<td></td>
<td>carcinogen—DNA adducts</td>
</tr>
<tr>
<td>Markers of proliferation</td>
<td>proliferating cell nuclear antigen</td>
</tr>
<tr>
<td></td>
<td>thymidine labeling index</td>
</tr>
<tr>
<td></td>
<td>bromodeoxyuridine incorporation</td>
</tr>
<tr>
<td></td>
<td>K67</td>
</tr>
<tr>
<td></td>
<td>ornithine decarboxylase</td>
</tr>
<tr>
<td>Markers of differentiation</td>
<td>blood group antigens</td>
</tr>
<tr>
<td></td>
<td>growth factors</td>
</tr>
<tr>
<td>Premalignant lesions</td>
<td>oral leukoplakia</td>
</tr>
<tr>
<td></td>
<td>bronchial metaplasia</td>
</tr>
<tr>
<td></td>
<td>sputum atypia</td>
</tr>
<tr>
<td></td>
<td>aberrant crypts</td>
</tr>
<tr>
<td></td>
<td>colonic adenomatous polyps</td>
</tr>
<tr>
<td></td>
<td>dysplastic nevi</td>
</tr>
</tbody>
</table>

Adapted from Kelloff et al. (157).
cancer risk, and alterations in DNA ploidy can often be seen long before actual cancer. Carcinogen–DNA adducts and carcinogen–protein adducts, often overlooked as potential intermediate markers, can be used to monitor the effectiveness of blocking agents in high-risk populations with known exposure to certain carcinogens.

Enhanced cellular proliferation is associated with neoplastic events. Cellular proliferation markers include proliferating cell nuclear antigen (PCNA), Ki67, incorporation of \[^{3}H\text{thymidine}\] or bromodeoxyuridine (BrdU) into DNA and ODC activity. PCNA, a 36 kDa auxiliary protein of DNA polymerase \(\alpha\), can be detected in proliferating cells in the G, S and G2 phases (164), while thymidine and BrdU labeling tend to label cells in S phase (165–167). Several non-carcinogenic phenomena can result in enhanced proliferation. For example, in the colonic mucosa, use of laxatives and the presence of ulcerative colitis will result in an expansion of the proliferative compartment (167). Markers of general cell proliferation may ultimately prove to be reliable intermediate endpoints in chemoprevention trials, particularly when used in conjunction with other markers, such as markers of cellular differentiation.

The expression of proteins, glycoproteins and complex carbohydrates can differ depending on the degree of cellular differentiation; such differences can, in some cases, be used to distinguish normal cells from neoplastic cells. Among the most widely studied differentiation markers are the blood group antigens. Tumor expression of antigen A has been found to be associated with a favorable prognosis in small-cell lung cancer (168). Antigens normally expressed only in the proximal colon (i.e. the A, B, H and Le\(^b\) antigens) can exhibit a loss of expression in cancers of the proximal colon or expression in the distal colon; the extended-chain versions of the Le\(^a\) and Le\(^b\) antigens have been found to be expressed preferentially in adenomatous polyps and frank colonic cancers compared with normal colonic mucosa (169). Results of a recent preliminary study conducted with 13-cis-retinoic acid showed that this retinoid modestly increased antigen A expression in oral leukoplakias (previously negative for type A) of subjects categorized as blood type A or AB (170).

Prior to the development of actual carcinoma in epithelial tissues, a histologically definable non-malignant stage termed intraepithelial neoplasia, can be found (171,172). Many such intraepithelial lesions as well as earlier, defined histologic lesions are now being evaluated and/or used as surrogates for cancer endpoints. Oral leukoplakia (white patches on the tongue or buccal mucosa) is associated with future oral cancer risk (173), and has been or is being used in several intervention trials (174). Bronchial metaplasia, the conversion of pseudostratified columnar epithelium to stratified squamous cell epithelium, is a condition linked with smoking (175). Aberrant crypts, colonic crypts that display increases in size, epithelial layer thickness and pericryptal areas, are being touted as a means of screening chemopreventative agents in rats (176) and are believed to be the earliest identifiable precursors of colon cancer (177). However, in one study that involved analysis of the effects of seven different diets on aberrant crypt formation and colon cancer in rats, aberrant crypt foci were found to be poorly correlated with the actual incidence of colon cancer (178).

This illustrates a major problem with the use of biomarkers as surrogate endpoints; the majority of biomarkers currently in use have not been rigorously validated (179,180). Thus far, there is no one universal biomarker, and there probably never will be, given the multifaceted nature of the collection of malignant neoplastic diseases that we term cancer. Despite this, many intervention trials are already underway utilizing these and other non-validated biomarkers rather than actual tumorigenic endpoints.

**Current human intervention trials**

There are numerous intervention trials underway in several different countries. In the USA, a principal sponsor of such trials is the Chemoprevention Branch of the National Cancer Institute (181,182). Over 30 trials (phases I–III) are currently being conducted; most of the phase III trials involve retinoids or \(\beta\)-carotene (182). Without question, one of the most ambitious trials is the Breast Cancer Prevention Trial, which began in 1992 in the USA and Canada. This trial will ultimately involve 16 000 women and is designed to test the ability of tamoxifen to inhibit breast cancer in women at high risk (183). The tamoxifen trial is not without controversy, since otherwise healthy women will be administered significant doses of a potent hormonal drug for years. Furthermore, tamoxifen has been found to be genotoxic (184), and induces hepatocellular adenomas and carcinomas in rats (185–187).

Some success has been achieved in many, albeit not all, chemoprevention trials. DeCosse et al. (188) reported the results of a study in which groups were given placebo, vitamins C and E or a high fiber supplement plus vitamins C and E. The results indicated a limited inhibitory effect of high fiber on large bowel neoplasia in patients with familial polyposis (188). An additional randomized trial conducted with vitamins C and E in an attempt to prevent recurrence of colorectal polyps found only a slight (and non-significant) reduction in recurrence with vitamin supplementation (189). Hong et al. (190) showed that isoretinoin inhibited the development of second primary tumors in patients treated for primary cancers of the head and neck. Garewal et al. (191) showed regression of oral leukoplakia in a single individual by administration of \(\beta\)-carotene for 3 months (191). Several studies have demonstrated the ability of the non-steroidal anti-inflammatory agent, sulindac, to cause the regression of colonic polyps (192–194). In a very recent study, Wang et al. (195) found no significant effect of calcium supplementation of \[^{3}H\text{thymidine incorporation, basal cell hyperplasia and esophageal dysplasia when used as surrogates for esophageal cancer.\n
**Obstacles to be overcome**

**Relative lack of participation by the pharmaceutical industry**

One might assume that the development of drugs for ultimate use by hundreds of thousands to millions of people in developed nations would involve massive contributions by the pharmaceutical industry. However, unlike the situation with the development of new chemotherapeutic agents, the development of candidate chemopreventative agents is proceeding with little support or input from the pharmaceutical industry. At this point, pharmaceutical support resides primarily in the supply of investigational agents to government sponsors (such as the National Cancer Institute) at no cost (196). While such efforts are laudable, the total commitment of the pharmaceutical industry worldwide probably represents 1% or less of the total financial resources that have been committed. The primary reasons for the lack of involvement of the pharmaceutical industry are economic in nature and thus, somewhat understandable: (i) many of the agents currently under trial in high-risk groups are GRAS compounds whose financial exploitation cannot be controlled by
any pharmaceutical interest; (ii) whether under patent or not, the cost of any potential chemopreventative agent must be minimal; (iii) the length of time required to prove actual efficacy of a given compound could extend to decades, allowing patents to expire prior to commercial distribution; (iv) the cost of the clinical trials that will ultimately be required to prove efficacy if cancer must be used as an endpoint; and (v) the potential legal ramifications (i.e., civil suits) that could be associated with long-term drug administration to individuals currently in good health.

**Lack of funding**

For 1990, in the USA alone, total costs associated with neoplastic diseases have been estimated at >$100 billion (197). The costs of actual treatment of cancer increase annually at a rate greater than inflation. A reduction in cancer incidence of just 10% would result in considerable savings. At this writing, the projected 1994 budget for the National Cancer Institute is ~$2 billion, of which only a fraction will be spent on cancer prevention and control of any kind (198). Clearly, the impressive advances made with chemopreventative agents in experimental models and the encouraging results of some of the clinical trials warrant an increased emphasis on funding for research in cancer chemoprevention. Those currently engaged in chemoprevention research must become more involved in funding decisions that will affect the field.

**Subject compliance and recruitment**

A number of issues concerning the prospective subjects of clinical chemopreventative trials warrant discussion. As any physician knows, patient compliance with acute self-administered dosing regimens can be less than adequate; it should come as no surprise that subject compliance with the chronic dosing regimens of chemopreventative clinical trials could be a considerable problem. Also, early withdrawal of subjects from multi-year protocols conducted at a single site can be a frequent occurrence in a highly mobile society such as the USA. Finally, recruitment of sufficient numbers of subjects for large-scale clinical trials can be quite difficult. There is increased interest in performing chemoprevention trials in countries such as China due to better compliance, lower mobility, greater genetic homogeneity and, often, expedited government approval. It is hoped that the studies that include such foreign subjects will be conducted primarily for the benefit of these individuals, rather than using such persons as mere surrogates (for western populations) that are more easily recruited, retained and prevented from legal retaliation.

**Conclusions**

Chemoprevention has earned serious consideration as a means of controlling cancer incidence as it is no longer merely a theoretical strategy, but an approach yielding continually more impressive experimental and clinical results. A number of identifiable groups at high risk for the development of cancer stand to benefit greatly from chemopreventative approaches. Several potent compounds have been developed that are capable of complete inhibition of tumorigenesis in experimental animal models. A more complete knowledge of the biologic effects of various chemopreventative agents should allow more rational combinations of such agents. Currently, the development of cancer chemoprevention as a discipline involves a relatively small number of scientists and clinicians. To ensure that cancer chemoprevention reaches its full potential, it is necessary to increase interest on the part of basic science and clinical researchers, encourage and enhance participation of the pharmaceutical industry and increase funding.

**References**


1744


