



Gary Ernest Smith. *Feed Lot*. Oil on canvas, 30" × 40". Courtesy of Raymond E. Johnson's Overland Gallery of Fine Art, Scottsdale, Arizona.

Lung cancer chemoprevention needs further development to decrease the incidence and mortality of this disease.

Progress in Lung Cancer Chemoprevention

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Background: Lung cancer is one of the major causes of cancer-related deaths. Lung cancer mortality figures argue powerfully for new approaches to control this disease. The term chemoprevention can be defined as the use of specific natural or synthetic chemical agents to reverse, suppress, or prevent premalignancy from progressing to invasive cancer.

Methods: Issues related to lung cancer chemoprevention are reviewed, including risk factors and identification of high-risk cohorts, endpoint biomarkers, and current and new chemopreventive agents. Also, important findings from chemoprevention randomized, controlled trials are summarized.

Results: Trials in lung cancer chemoprevention have so far produced either neutral or harmful primary endpoint results, whether in the primary, secondary, or tertiary settings. Lung cancer was not prevented by beta-carotene, alpha-tocopherol, retinol, retinyl palmitate, N-acetylcysteine, or isotretinoin in smokers. Secondary results from the phase III trials involving selenium and vitamin E, as well as results from the US Intergroup NCI I91-0001 trial supporting treatment with isotretinoin in never and former smokers, are promising and may help define new avenues for chemoprevention.

Conclusions: The concept of chemoprevention in lung cancer is still in its infancy but one day may have a significant impact on the incidence and mortality of this leading cancer threat. Molecular markers of risk, drug activity and targeting, improved imaging techniques, and new drug delivery systems are being evaluated.

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Introduction

Lung cancer is the most common cause of cancer mortality worldwide, with over 1.3 million deaths and continues to be a major health problem. In the United States, the disease has been the leading cause of cancer in men for years, and since 1988 it has also become the number one cause of cancer death in women. It is estimated that in the year 2002, approximately 169,400 people will be diagnosed with lung cancer and 154,900 will die of the disease, surpassing the combined death rates of breast, prostate, and colon cancers.¹

Aggressive local control by surgical resection and/or radiation ablation is currently the mainstay of lung cancer therapy for early-stage disease. Systemic chemotherapy has been used in an attempt to prolong symptom-free survival in patients deemed unresectable or in those with metastatic disease. These interventions have produced slight declines in mortality rates in recent years; however, it appears unlikely that additional marked improvements with these practices alone will occur in the near future.^{2,4} This grim overview argues powerfully for new, emerging approaches such as biologic or molecularly targeted therapy and chemoprevention for controlling lung cancer. Chemoprevention first described by Sporn⁵ in 1976 can be defined as “the use of specific natural or synthetic chemical agents to reverse, suppress or prevent carcinogenic progression to invasive cancer.” This review focuses on several issues related to lung cancer chemoprevention, including risk factors and identification of high-risk cohorts, endpoint biomarkers, and current and new chemopreventive agents. Also, the results of clinical chemoprevention trials are reviewed.

Risk Factors

Several risk factors that are associated with an increased risk of developing lung cancer have been identified, such as cigarette smoking, airflow obstruction, and exposure to asbestos, radon, arsenic, ionizing radiation, haloethers, polycyclic aromatic hydrocarbons, and nickel. Potential roles for previous pulmonary parenchymal scarring, familial factors and dietary risk factors are less well established. Cigarette smoking is estimated to be responsible for approximately 87% of lung cancer cases, and evidence for this link is indisputable.⁶ Estimates of the relative risk of disease in the long-term smoker compared with the lifetime nonsmoker vary from 10- to 30-fold. The cumulative lung cancer risk among heavy smokers may be as high as 30% compared with a lifetime risk of 1% or less in nonsmokers.^{7,8} The risk of carcinoma increases with the number of cigarettes smoked, years of smoking, earlier

age of onset, degree of inhalation, tar and nicotine content, and use of unfiltered cigarettes. Additionally, risk decreases proportionally with the number of years after quitting.⁹ Despite the reduction in lung cancer risk observed with smoking cessation, several studies have demonstrated that former smokers still have a higher lung cancer risk than nonsmokers.¹⁰⁻¹² Even 10 years after smoking cessation, the risk was still significantly elevated.¹³ Consequently, former smokers account for a large proportion of lung cancers in the United States. Analyses from M. D. Anderson Cancer Center and Harvard-affiliated hospitals showed that more than 50% of lung cancer cases occur in former smokers.^{14,15}

Not all persons who are exposed to cigarette smoke and other potentially carcinogenic environmental influences develop lung cancer. The evaluation of predisposing factors for the disease — mainly by ecologic or case-control studies, prospective population-based research, and familial aggregation studies — has produced little success in identifying the characteristics that single out patients who possess the highest smoking-related risk. This is regrettable since the ability to identify smokers with the highest risks of developing tobacco-related cancers has substantial implications for the success of chemopreventive interventions. More recently, the molecular epidemiology approach with the confluence of sophisticated advances in molecular biology and field-tested epidemiologic methodology has enhanced our knowledge of tobacco carcinogenesis and susceptibility. A major area of interest has focused on the interactions between tobacco carcinogens, genetic polymorphisms involved in activating and detoxifying these carcinogens, and host-cell efficiency in monitoring and repairing tobacco carcinogen-DNA damage. As an example, a recent study reported a relationship between environmental tobacco smoke exposure and increasing lung cancer risk among nonsmoking women with a common genetic deficiency in glutathione S-transferase M1 (GSTM1) enzymatic activity because of a genetic polymorphism in the GSTM1 gene.¹⁶ Glutathione S-transferase M1 is believed to play a role in detoxifying carcinogens in tobacco smoke; thus, mutations that decrease its activity could serve to promote tumorigenesis. Although endowed with this fresh information about lung cancer susceptibility, it is still not possible to identify those individuals at increased risk.

To help with this effort, the National Cancer Institute has formed and developed the Prevention Trials Decision Network (PTDN), which helps to formalize the evaluation and approval process for large-scale chemoprevention trials and addresses large trial prioritization and the associated issues of risk modeling, identification, and validation of biomarkers and chemopreventive

agent selection and development.^{17,18} Cohorts who are at high risk for cancer according to the PTDN criteria include those with the following attributes: (1) a genetic predisposition to cancer, including polymorphisms of common genes that affect how an individual responds to an environmental insult or cancer-predisposing genetic mutations, (2) exposure to known carcinogens, and (3) the presence of positive markers of increase risk for certain cancers. Using the PTDN criteria strategy, it is hoped that a quantitative risk model that incorporates biomarkers of risk could be developed and subsequently validated in prospective, randomized, controlled studies performed on large series of patients. The acquisition of such a model will prove useful for lung cancer chemoprevention trials, paralleling the Gail model and its successful employment in the NASBP-P1 phase III breast cancer chemoprevention trial.^{19,20}

Lung Cancer Carcinogenesis and Chemoprevention

Lung carcinoma appears to develop from a pluripotent stem cell involved in the generation of the bronchial epithelium and capable of differentiation along several pathways.² The biology of this process is based on two themes: *field cancerization* and *multistep carcinogenesis*.²¹

Field carcinogenesis denotes diffuse epithelial injury resulting from carcinogenic (eg, tobacco smoke) exposure in an entire epithelial field or region, setting off a chronic pattern of tissue damage and wound healing where changes can be detected at the gross, microscopic, and molecular levels. The clinical importance of this phenomenon is best illustrated in aerodigestive cancers for which both synchronous and metachronous second primary tumors are common.

Chronic carcinogenic insult sets off a multistep process characterized by the occurrence of initiation, promotion, and progression events occurring over latent periods of a decade or more. These events produce an accumulation of genetic and epigenetic alterations of at least three groups of genes: proto-oncogenes, tumor suppressor genes, and mutator genes resulting in imbalances between cellular proliferation, apoptosis, and shedding.² Imbalance in cellular population kinetics promotes a build-up of cells that, if sufficiently abnormal, have malignant capability. Numerous systems including repair, replacement or recruitment, replication, and redundancy mechanisms become operational to help restore structural and functional integrity. In some instances, however, these mechanisms fail or are overwhelmed, and unrepaired injury not only occurs but also is propagated, resulting in the trigger-

ing of a transformation from normal to premalignant cells and eventually to frank invasive carcinoma.

The essence of chemoprevention is intervention within this multistep carcinogenic process. Using pharmacologic or natural compounds, chemoprevention aims to block, reverse, or inhibit this process by blocking DNA damage, retarding or reversing malignant phenotype, or inducing apoptosis in the damaged cells of premalignant lesions.²² General chemoprevention is divided conceptually into three settings: primary, secondary, and tertiary prevention. Primary prevention is defined as an intervention intended to delay or prevent the development of initial cancer in healthy individuals (eg, high risk). Approaches such as smoking prevention and cessation treatments or the use of drugs in a group of asymptomatic smokers are examples of this strategy. Secondary prevention is aimed at persons with premalignant conditions, and tertiary prevention involves decreasing the morbidity of established disease. Chemoprevention of second primary cancer in patients cured of an initial cancer is a good example of tertiary prevention.

Surrogate Endpoint Biomarkers in Lung Cancer

Surrogate endpoint biomarkers (SEBs) are used as intermediate indicators of cancer incidence reduction in chemoprevention studies. To be most useful, SEBs are required to be integrally involved in the process of carcinogenesis such that the changes of expression correlate highly with disease course. Markers must be differentially expressed in normal and premalignant or high-risk tissue. They must also occur in sufficient amounts to permit their biological and statistical assessment, assayed dependably and quantitatively, and measured without difficulty. Lastly, their expression should be able to be modulated by efficacious chemopreventive interventions but not vary spontaneously or have an appreciable spontaneous remission rate.^{21,23,24}

At present there are no validated SEBs for lung cancer in the context of chemoprevention trials with cancer incidence reduction as the definitive endpoint. Therefore, their development and study are extremely important. The justification for using surrogate endpoints includes such factors as reduced sample size and shorter time scale for studies. The number of potential SEBs is substantial (Table 1) and is expanding with the advances in the field of genetics.

Cytologic and histopathologic markers include nuclear features, nucleolar features, and tissue architecture. Nuclear features of interest include grade, shape, area, texture (reflecting chromatin stippling), nuclear

polymorphism, and ploidy. Nucleolar features of interest include size, shape, and position. Tissue architectural measurements make use of the finding that disordered nuclei are crowded and irregular. These markers are now being quantified using stoichiometric stains viewed by computer-assisted imaging systems. Quantitative cytology and histopathology allow for an objective, reproducible measure of what is observed by the pathologist. A class of biomarkers of increasing importance assesses proliferation and growth regulation and includes retinoic acid receptor β (RAR β) and other retinoid receptors, proliferating cell nuclear antigen (PCNA), Ki-67, transforming growth factor β (TGF β), and epidermal growth factor receptor (EGFR). Other markers such as the genomic instability markers may be the most important biological markers of all, possibly reflecting the sum of the changes in all other categories. DNA abnormalities (eg, DNA hypomethylation, loss of heterozygosity [LOH], point mutations, gene amplification) and chromosome aberrations (micronuclei from chromosomal damage, chromosomal polysomy, and deletions at 3p, 5q, 9p, 11q, 13q, and 17p)

Table 1. — Selected Examples of Intermediate Endpoint Chemoprevention Biomarkers

Histopathologic
Squamous dysplasia
Carcinoma in situ
Atypical alveolar hyperplasia
Cellular
Proliferation:
Ki-67 antigen expression
Proliferating cell nuclear antigen
Differentiation:
Loss of high molecular weight cytokeratins (50-64 kD)
Altered blood group antigens (Lewis antigen)
Retinoic acid receptors
Lectin labeling
hnRNPA2/B1
Apoptosis:
Bcl-2/bax
Biochemical
Circulating insulin-like growth factor (IGF-1)
Genetic
Ras mutations (K-ras)
<i>c-erbB</i> proto-oncogenes (<i>erbB1</i> or EGFR; <i>erbB2</i> (HER2/neu)
<i>c-myc</i>
p53
Chromosomal loss or gain (eg, 3p, 5q, 9p, 11q, 13q, 17p)
Molecular
DNA methylation:
Promotor CpG island methylation (p16, DAPK, ECAD, MGMT, GSTP1)
DAPK = death-associated protein kinase
ECAD = E-cadherin
EGFR = epidermal growth factor receptor
GSTP1 = glutathione S-transferase P1
hnRNPA2/B1 = heterogenous nuclear ribonucleoprotein A2/B1
MGMT = O ⁶ -methylguanine-DNA-methyltransferase

have been proposed as promising markers for lung cancer trials. Because of the complexity and multifaceted nature of carcinogenesis, it is unlikely that any one of these markers alone will be able to encapsulate all the information necessary to be a viable endpoint. A panel of markers probably will be required to gather sufficient information to assess the effects of preventive agents and perhaps one day to function as a valid endpoint for cancer prevention trials.

Validation of these markers that predict for clinical benefit or risk reduction in randomized, controlled trials will be critical to the progress of lung cancer chemoprevention. Detailed discussions of the statistical issues involved in this process have been published elsewhere.^{25,26} Once credentialed, these markers can accelerate new agent registration by serving as the primary study endpoint in phase IIB and phase III clinical trials. It is anticipated that incorporation of intermediate markers will enable efficient, small (50 to 200 subjects), proof-of-principal trials and medium-size (300 to 500 subjects) definitive clinical trials.

Chemopreventive Agents

Agent requirements include experimental or epidemiologic data showing chemopreventive efficacy, a mechanistic rationale for the chemopreventive activity observed, and safety on chronic administration. Currently, numerous chemopreventive agents are in various stages of development. This variation in research phase and the necessity for additional study clarifying mechanisms of action make it challenging to design a unifying and widely accepted classification system for these agents. Some agents, including the micronutrients or antioxidants and the retinoids, are for general cancer chemoprevention and show activity in many sites. Others are far more specific, having site-specific molecular targets. A partial list of these agents appears in Table 2. Drugs that have been tested in patients for lung cancer prevention include all-*trans*-retinoic acid (ATRA), 13-*cis*-retinoic acid or 9-*cis*-retinoic acid, retinyl palmitate, fenretinide (*N*-[4-hydroxyphenyl] retinamide), beta-carotene, alpha-tocopherol, and selenium.

Retinoids

Retinoids refer to natural or synthetic compounds that share a similar chemical structure consisting of a cyclic end group, a polyene side chain, and polar end group. They have complex biological effects, including modulation of differentiation,^{27,28} proliferation, apoptosis, and immune status with both normal and neoplastic tissues. This complexity is a function of not only the diversity of the retinoid ligands, but also the variety of

nuclear receptors that mediate their activity.^{27,28} There are two major classes of retinoid receptors: nuclear RARs and retinoid receptors (RXRs). Each receptor contains alpha, beta, and gamma subtypes, and several of these subclasses have multiple isoforms produced through differential promoter usage and alternative splicing of receptor transcripts.²⁸⁻³⁰ RXRs are active only as heterodimers or homodimers. RXRs can form homodimers or heterodimers by binding with RARs or a host of other receptors, all of which are members of the steroid hormone superfamily of receptors. RARs form only heterodimers and only with RXRs. These different receptor dimerizations confer effector specificity to dif-

ferent cells. Each receptor is thought to bind to specific response elements named retinoic acid response elements (RAREs) that govern the expression of genes and modify posttranscriptional mechanisms.^{27,28}

Current systemic therapy with retinoid compounds is limited by substantial toxicities that result from activation of multiple signaling pathways. These toxicities involve numerous systems, including the skin and mucosa (dryness, desquamation, peeling, pruritus, dermatitis, and cutaneous photosensitivity), liver (reversible elevations of hepatic enzymes), skeleton (ligament calcification, skeletal hyperostosis), central nervous system (headache), and reversible abnormalities in serum lipids.^{31,32} Occasionally, many of these side effects can be ameliorated by the concomitant use of alpha-tocopherol without any loss of retinoid activity.³³ Whereas overall toxicity may be less with fenretinide, this retinoid has the additional adverse effect of impaired visual adaptation to darkness (“nightblindness”), an effect that appears to be related to the lowering of retinol levels.³⁴ This reversible ocular toxicity of fenretinide occurs in approximately 25% of patients, is asymptomatic in 50% of affected patients, and can be averted or minimized by allowing drug holidays.^{34,35}

More than 1,000 retinoids have been synthesized. Current efforts are concentrating on the development of receptor-selective and function-selective retinoids through molecular targeting strategies and structure activity relationship studies based on binding and transactivation assays. Primary targets and related examples of receptor-selective retinoid agents currently include RAR α /AM80, RAR β /CD2317, RAR γ /CD437, RXR/LG268, and the pan-agonist RARs/LGD1550.³⁶ So far, these strategies have proved successful in helping to generate retinoid agents that offer not only good efficacy but also good tolerability.

Carotenoids

Carotenoids constitute a class of over 600 compounds found predominantly in fruits and vegetables. Of these compounds, beta-carotene has been the most extensively studied. This molecule has been reported to have a number of actions including important antioxidant activity and enhancement of immune function. Original epidemiologic data showed a correlation between low dietary beta-carotene and increased risk of lung cancer; however, clinical trials involving supplementation of pharmacologic doses revealed detrimental effects. Possible explanations for this effect include an inhibition of absorption of other nutrients by large doses of beta-carotene and the autocatalytic pro-oxidant activity of beta-carotene under high oxygen tension such as that occurring in the

Table 2. — Selected Examples of Potential Chemopreventive Agents

Retinoids:
9- <i>cis</i> retinoic acid
13- <i>cis</i> retinoic acid
fenretinide
all- <i>trans</i> -retinoic acid
bexarotene (Targretin)
Carotenoids:
lycopene
Soy Isoflavones:
genistein
daidzein
NSAIDs and Related Agents:
R-flurbiprofen
sulindac sulfone
selected cyclooxygenase-2 inhibitors (celecoxib, rofecoxib)
Green Tea Polyphenols:
green tea extract
epigallocatechin-3-gallate
Inhibitors of Polyamine Biosynthesis:
alpha-difluoromethylornithine
Lipoxygenase Modulators
Protein Kinase C Inhibitors
Matrix Metalloproteinase Inhibitors
Farnesyltransferase Inhibitors:
SCH66336
R115777
Tyrosine Kinase Inhibitors:
epidermal growth factor receptor inhibitors
- monoclonal antibodies (C225)
- small molecules (ZD1839, OSI-774)
Monoterpenes:
perillyl alcohol
limonene
Antioxidants:
selenium
vitamin E
Histone Deacetylase Inhibitors
Demethylating Agents:
5-aza-C
Gene-Based Interventions:
p53 adenovirus

lungs of smokers.³⁷⁻³⁹ Regardless of the mechanism, given the absence of beneficial effects and the potential for harm, this agent is now being avoided in heavy smokers and is no longer being used for lung cancer chemoprevention.

In spite of the negative effects from beta-carotene, the carotenoids as a family continue to be of interest as potential prevention agents. Stahl and Sies⁴⁰ examined the biochemistry and biophysics of lycopene, a naturally occurring hydrocarbon found in tomatoes and their products. Lycopene is a potent antioxidant, and its consumption has been associated with a lower lung cancer risk. In vivo animal trials assessing its chemopreventive effects in a multiorgan carcinogenesis model found pulmonary adenoma and carcinoma formation were reduced with lycopene.^{41,42} Before large intervention trials can be justified, however, small human trials will need to be performed to determine if the agent has biologic activity and toxicity.

Alpha-Tocopherol

Alpha-tocopherol, the predominant form of vitamin E, is also a putative antioxidant. Vitamin E has been shown to have potent inhibitory activity on cell proliferation in various cancer cell lines including lung cancer, and high-molecular-weight DNA analysis revealed fragmentation consistent with apoptosis.⁴³ However, epidemiologic studies have failed to show a significant association with lung cancer risk. Its efficacy in the chemoprevention of lung cancer has not been demonstrated, and further evaluation in prevention trials is needed before firm recommendations can be made. In limited studies, vitamin E had possible protective effects on other cancers.

Selenium

Selenium is a component of the oxidative enzyme glutathione peroxidase. The proposed mechanisms of action of this micronutrient include antioxidant defense, anticarcinogen, antiproliferation, and pro-apoptosis. Selenium as L-selenomethionine has been shown to inhibit cell growth, induce apoptosis in vitro, and retard carcinogenesis at higher dose levels in animal models. Epidemiologic data suggest an inverse relationship between selenium intake and lung cancer.⁴⁴ A recent major clinical trial stimulated further interest in the role of selenium in chemoprevention. In a study by Clark et al⁴⁵ designed to determine selenium effects on the incidence of skin basal or squamous cell carcinomas, nutritional supplementation with this agent showed no consequences on skin cancer incidence; however, secondary analyses revealed that it was associated with significantly fewer lung cancers. These

observations led to an ongoing intergroup trial examining the effects of selenium on the reduction of lung cancer-associated second primary lung tumors (SPTs).

Other Potential Agents

Several other agents being considered for the chemoprevention of lung cancer include the monoterpenes limonene and perillyl alcohol, the isoflavone genistein (which is found in high concentrations in soybeans and soy products), the lipoxygenase and cyclo-oxygenase inhibitors, demethylating agents, and the signal transduction modulators (EGFR inhibitors and farnesyltransferase inhibitors). The latter appear to be especially promising and are currently under clinical trial.

Preclinical Testing of Suitable Agents

The preclinical development of suitable chemoprevention agents includes a preliminary assessment of their efficacy using in vitro and cell-based mechanistic assays and in vivo screens in well-established, chemically induced, whole-animal tumor models. Potential inhibitors of lung carcinogenesis are evaluated using models developed in both hamster and mouse species. Of the hamster lung cancer models, one model utilizes the direct-acting carcinogen methylnitrosourea, and the other utilizes diethylnitrosamine.⁴⁶ Tumors appear within 6 months, and both models are responsive to modulation by several classes of potential chemopreventive drugs. Another practical and useful model for the study of lung cancer prevention is the A/J mouse model that reliably produces lung tumors with an oncogenic *K-ras* mutation when induced by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, a tobacco-related carcinogen.⁴⁷ These models afford a strategic framework for evaluating agents according to defined criteria and not only provide evidence of efficacy, but also serve to generate important dose-response, toxicity, and pharmacokinetic data required prior to early-phase clinical testing in humans.

Completed Randomized, Controlled Trials

Primary Chemoprevention

Two large National Cancer Institute (NCI)-sponsored chemoprevention trials with lung cancer as a primary endpoint have been completed. The Alpha-Tocopherol Beta-Carotene Cancer Prevention Study Group⁴⁸ used a 2 × 2 factorial design to test alpha-tocopherol and beta-carotene in 29,133 Finnish chronic male

smokers aged 50 to 69 years. Subjects were randomized to 1 of 4 groups: alpha-tocopherol 50 mg/day alone, beta-carotene 20 mg/day alone, both alpha-tocopherol and beta-carotene at the above doses, or placebo. They were followed for 5 to 8 years. Unexpectedly, subjects receiving beta-carotene either alone or in combination with alpha-tocopherol had an 18% increase in the incidence of lung cancer (relative risk [RR] = 1.18; 95% confidence interval [CI] 1.03-1.36; $P=.01$) and an 8% increase in total mortality ($P=.08$). There appeared to be a stronger adverse effect from beta-carotene in men who smoked more than 20 cigarettes a day, thus raising the serious issue that pharmacologic doses of beta-carotene could potentially be harmful in active smokers.

The Beta-Carotene and Retinol Efficacy Trial⁴⁹ confirmed the results of the Finnish trial. This randomized, double-blinded, placebo-controlled trial tested the combination of 30 mg of beta-carotene and 25,000 IU of retinyl palmitate against placebo in men and women aged 50 to 69 years at high risk for lung cancer development. Among the 18,314 participants, 14,254 individuals had at least a 20 pack-year smoking history and were either current or former smokers, and 4,060 men had extensive occupational exposure to asbestos. This trial was stopped after 21 months because no benefit and possible harm were seen. Lung cancer incidence increased by 28% in the active intervention group (RR = 1.28; 95% CI 1.04-1.57; $P=.02$). Overall mortality also increased 17% in this group ($P=.02$).

The above findings showing harmful effects of beta-carotene were by no means universal. The Physicians' Health Study,⁵⁰ a randomized, double-blinded, placebo-controlled trial, studied 22,071 healthy male physicians and randomized them to either 15 mg of beta-carotene on alternate days vs placebo. The use of supplemental beta-carotene in this study showed no adverse or beneficial effects on cancer incidence or overall mortality during a 12-year follow-up.

Secondary Chemoprevention

Several lung chemoprevention trials used progressive histologic changes in the bronchial epithelium as a study endpoint. A total of five randomized trials have been conducted. Collectively, these results demonstrated that retinoids added no significant benefit to the effects of smoking cessation in the reversal of squamous metaplasia or dysplasia (Table 3).⁵¹⁻⁵⁵ One study showed improvement of sputum atypia in smokers with folate and vitamin B₁₂ supplements.⁵⁵ This result

Table 3. — Premalignancy Trials in Lung Cancer

Trial	Intervention	Endpoint	No. of Patients	Outcome
Lee ⁵¹	Isotretinoin	Metaplasia	40	Negative
Kurie ⁵²	Fenretinide	Metaplasia	82	Negative
Arnold ⁵³	Etretinate	Metaplasia	150	Negative
McLarty ⁵⁴	Beta-carotene/retinol	Sputum atypia	1,067	Negative
Heimburger ⁵⁵	Vitamin B ₁₂ /folic acid	Sputum atypia	73	Positive*

* A reanalysis of the data using standard analytical methods found a negative outcome.²¹

is questionable, however, because of the small sample size, substantial spontaneous and inter-observer variability in atypia assessments, and complex and non-standard statistical analytical methods. A reanalysis of these data using standard analytical methods found no significant difference between the two groups.²¹

Tertiary Chemoprevention

Patients diagnosed with a first primary cancer have a high incidence of SPTs. These tumors must be distinguished from recurrent lesions and are defined as (1) a new cancer of a different histologic subtype, (2) a cancer, regardless of site, that occurs after an interval of more than 3 years, or (3) a cancer presenting as a solitary mass that is of squamous cell histologic type, develops within 3 years, and occurs in the absence of local or regional disease accompanied by evidence of dysplasia or carcinoma in situ within the bronchial epithelium.⁵⁶ Three definitive phase III studies evaluating retinoids in lung SPT prevention have been completed. The scientific rationale for testing retinoids in this setting came largely from head and neck chemoprevention data showing that these agents significantly reversed oral premalignancy and prevented SPTs.

In the first study, the adjuvant effect of high-dose retinyl palmitate (300,000 IU per day) was evaluated in 307 patients with early-stage lung cancer, randomly assigned after surgical resection to active drug or no further treatment.⁵⁷ After a median follow-up of 46 months, the SPT rate was 39% in the retinyl palmitate arm and 48% in the no-treatment control arm. A statistically significant difference in favor of treatment was observed regarding time to SPT development within the aerodigestive field.

These encouraging results were followed by a European study, the European Study on Chemoprevention with vitamin A and *N*-acetylcysteine (EUROSCAN). This trial was an open-label multicenter trial of retinyl palmitate and *N*-acetylcysteine (an aminothioli precursor of reduced glutathione) for 2 years in a 2 × 2 factorial design in 2,592 patients.⁵⁸ The endpoint was SPT

prevention following treatment with curative intent of early-stage head and neck cancer or lung cancer. Results showed no difference between the placebo group and the three active treatment arms for SPTs, event-free survival, or long-term survival rates.

The last of these trials carried out through the Oncology Intergroup involving all NCI Cooperative Oncology groups studied the efficacy of isotretinoin in the prevention of SPT after complete resection of stage I non-small-cell lung cancer (US Intergroup NCI I91-0001). In this randomized, double-blinded, placebo-controlled trial, more than 1,000 patients received 3 years of intervention and an additional 4 years of follow-up.⁵⁹ Time to SPT was the primary endpoint. Additional study objectives were to look at the qualitative and quantitative toxicity of daily low-dose of the retinoid and compare the overall survival rates of the two groups. After a median follow-up of 3.5 years, no statistically significant differences were observed with respect to time to SPTs, recurrences, or mortality. Secondary multivariate analyses suggested that isotretinoin was harmful in current smokers and beneficial in never smokers. Possible reasons for this finding include potential adverse interactions of retinoic acid with tobacco smoke. For example, tobacco carcinogens can suppress RAR β expression and can induce retinoic acid metabolism and DNA methylation. Retinoic acid and smoking can increase gastrin-releasing peptide (GRP) expression and smoking can increase GRP receptor expression. Finally, the tobacco carcinogen benzo[*a*]pyrene and retinoic acid can induce NF- κ B activation. These smoking-related genetic and epigenetic changes are more dominant in the lungs of active smokers than in the lungs of former smokers, which may help explain the difference in recurrence between these two subgroups. The possible persistence of these changes in the lungs of former smokers may help to explain the difference in recurrence between former and never smokers.⁵⁹ Although this finding of a differential retinoid-smoking interaction appears to be important, it must be viewed as exploratory at best, given the limitations of unplanned retrospective analyses and the high likelihood of type I and type II errors.

More recently, Mayne et al⁶⁰ reported the results of a randomized trial evaluating the efficacy of supplemental beta-carotene on reducing failure attributable to SPTs (head and neck, esophagus, and lung) and local recurrences in patients curatively treated for early-stage cancers of the head and neck. Patients were recruited from the state tumor registry, a procedure that may eventually serve as a model for future chemoprevention trials, and were randomly assigned to receive 50 mg of beta-carotene per day or a corresponding identical placebo. After a median follow-up of just over 4

years, no statistically significant differences were detected between the two groups. In site-specific analyses, supplemental beta-carotene had no significant effect on second head and neck cancer or lung cancer; however, the point estimates suggested a possible decrease in second head and neck cancer but a likely increase in lung cancer risk. These results, if reproduced in already completed or future studies, could serve as an important starting point for mechanistic studies addressing differential responses of chemopreventive interventions in one epithelial site vs another.

Ongoing Trials

Several large trials are testing chemoprevention of many cancers. In the United States, the Lung Cancer Biomarkers Chemoprevention Consortium is preparing to launch two novel chemoprevention projects called SPORE (Specialized Programs of Research Excellence) Trials of Lung Cancer Prevention (STOP). These projects will take place at selected institutions across the country with additional investigational centers recruited based on patient accrual. The trials are randomized, phase IIb studies designed to evaluate the effects of R115777, a farnesyltransferase inhibitor (STOP-FTI) and ZD1839, a tyrosine kinase inhibitor (STOP-TKI) in former and current smokers with a previous specified smoking-related cancer. Patient eligibility requirements and trial conduct are identical in the two studies, and borrowing of control groups is employed to minimize overall sample size. The major objectives are to evaluate the effectiveness of these compounds in the response of histology and modulation of the Ki-67 labeling index and to assess intermediate serological and tissue markers as preliminary predictors for efficacy.

Conclusions

All the prospective, randomized, controlled trials in lung cancer chemoprevention have so far produced either neutral or harmful primary endpoint results, whether in the primary, secondary, and tertiary settings. The data suggest that lung cancer was not prevented by beta-carotene, alpha-tocopherol, retinol, retinyl palmitate, *N*-acetylcysteine, or isotretinoin in smokers. Secondary results from the phase III trials involving selenium and vitamin E, as well as results from the US Intergroup NCI I91-0001 trial supporting treatment with isotretinoin in never and former smokers, present a promising direction for future clinical study. Other areas of promise for future study involve molecular markers of risk and drug activity and molecular targets for new drug development, improved imaging techniques, and new drug delivery systems.

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