Intermediate Markers and Molecular Genetics of Lung Carcinogenesis

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Identification of intermediate endpoint markers that parallel lung cancer progression may enhance the efficacy of specific interventions.

Background: Various options are available for the local control of cancer in the breast - mastectomy, conservation therapy, and mastectomy with reconstruction.

Methods: To evaluate the benefits and drawbacks of the available management options, the authors combine their extensive experience with a review of the literature on outcomes from these approaches.

Results: Conservation therapy provides survival outcomes similar to those from mastectomy. Differences in local recurrence rates can be minimized by close adherence to guidelines for patient selection, operative approach, and radiation technique.

Conclusions: The role of the physician in selecting a local therapy for breast cancer has changed from one of informing the patient of the treatment to assessing the presence of medical contraindications to any of the treatments, educating the patients on each treatment approach, providing access to multidisciplinary consultation, and allowing the patient to choose an appropriate treatment approach.

Tobacco drieth the brain, dimeth the sight, vitiateth the smell, hurleth the stomach, destroyeth the concoction, disturbeth the humors and spirits, corrupteth the breath, induceth a trembling of the limbs, exsiccateth the windpipe, lungs, and liver, annoyeth the milt, scorcheth the heart, and causeth the blood to be adjusted.

Tobias Venner (1577-1660)

Introduction

Cancer is the summation of a complex series of molecular events leading to dysregulated growth and altered functional capacities.1-2 The lengthy and cumulative nature of these events has led to the recognition that focusing on the process of carcinogenesis rather than in attempting to cure disseminated cancer may be the more relevant way to approach patients with this disease.3 Whereas local modalities (eg, surgery, radiation therapy) have been the mainstay of treatment of most epithelial cancers, the high systemic relapse rates for even early-stage common cancers originating in the breast, colon, and lung have led to the development of adjuvant chemotherapeutic approaches to complement definitive local therapies.4,5 In the case of lung cancer, however, little benefit has been realized from such additional approaches, and the unacceptably high mortality rate associated with the diagnosis of lung cancer has not changed appreciably over the past several decades.6 This underscores the need for a different approach to these patients -- one that focuses on prevention of future invasive disease rather than on attempts to cure current disseminated disease.

Recent clinical studies with pharmacologic chemopreventive agents administered to patients at high risk for development of cancer offer promise in arresting neoplastic progression.7 The practical issue for clinical trials becomes how to define and follow response to these agents. Traditional endpoints in cancer clinical trials have included cancer-related mortality or changes in measurable disease. In the case of chemoprevention trials, however, measurable disease is not present and only a fraction of the population will ever develop cancer (usually years later). Economic and temporal constraints mandate the identification of alternative endpoints for such trials.

The concept of using intermediate endpoint markers to manage patients at risk for disease is not new to medical practice. Blood pressure and cholesterol measurements and their normalization through a variety of clinical interventions are part of routine health maintenance for a large segment of the population. Since high blood pressure is known to be a major risk factor for cardiovascular disease, clinical testing of new antihypertensive agents uses blood pressure measurement rather than myocardial infarction as an endpoint.

Similarly in oncology, cervical dysplasia diagnosed by Papanicolaou smear, with its high rate of progression to invasive cancer, has been used as a surrogate for invasive cancer and forms the basis for clinical therapy. Since the progression from dysplasia to carcinoma in situ to invasive cancer is well established for cervical cancer, one can intervene before invasive cancer occurs. Thus, the rationale for early intervention based on intermediate endpoint markers is predicated on two factors: (1) a very tight linkage must exist between the intermediate endpoint (ie, blood pressure or cervical dysplasia) and the final outcome (ie, cardiovascular disease or cervical cancer, respectively) and (2) intervention at an early stage must be feasible and more efficacious and/or easier (ie, less extensive surgery) than at the late, overt-
In the case of lung cancer, a clear need exists for identifying alternative approaches toward earlier diagnosis and intervention. In 1997 alone, 178,100 new cases and 160,400 deaths are expected to be attributed to lung cancer. With an 87% mortality associated with the diagnosis of lung cancer, that is primarily due to advanced stage at diagnosis, intervention during the process of carcinogenesis -- before the development of invasive disease -- is mandatory. In contrast to cervical cancer, however, lung cancer consists of a variety of histologies ranging from small-cell lung cancer to several subtypes of non-small-cell lung cancer (eg, squamous cell, adenocarcinoma, large-cell carcinoma), and the preinvasive precursor lesions have not been identified for all the subtypes. Nevertheless, the increasing understanding of the molecular biology of lung cancer offers a variety of molecular candidates for intermediate endpoint analysis. This review focuses on non-small-cell lung cancer biology and its application to chemoprevention clinical trials.

**Epithelial Carcinogenesis: Field Cancerization Occurring in Multiple Stages**

It has long been recognized that epithelial carcinogenesis proceeds through multiple distinct stages, each characterized by specific molecular and cellular alterations. As expressed schematically in the Figure, these stages have been identified as (1) initiation, the rapid, irreversible damage to a cell and its progeny by a carcinogen, (2) promotion, the lengthy reversible growth stimulation of the initiated clones, and (3) progression, the later, generally irreversible events leading to invasive malignant disease. Cytogenetic and molecular biology analyses show that the majority of genetic alterations occur during the progression phase. The concept of prevention of tumor progression in the exposed epithelium, the natural corollary is to focus on the early carcinogenic events occurring in that epithelium both as targets for intervention and intermediate markers for these trials.

The implications of field cancerization are not only that the presence of multiple concurrent preneoplastic foci makes the surrounding lung a rich source of precursor lesions for small lesions to study for genetic abnormalities, but also that local therapies aimed at excising an established invasive cancer are insufficient to prevent future disease. This is illustrated by recent studies showing that patients with multiple lung cancers for abnormalities in p53, K-ras, and chromosome 3p. The results of these studies showed different patterns of mutations in histologically distinct lesions from the same patients, again confirming the independent origin of these multiple lesions. The sequential acquisition of genetic alterations during carcinogenesis is termed clonal evolution. In the case of colon carcinogenesis, the work of Fearon and Vogelstein has demonstrated increasing mutations and allelic losses involving a variety of genes during the progression from adenoma to carcinoma. The existence of well-characterized precursor lesions to colonic adenocarcinoma facilitated the molecular characterization and temporal ordering of these molecular abnormalities during the multistage carcinogenesis process. It has become increasingly evident, however, that the acquisition of abnormalities in a specific order may be less important than the total number of mutations acquired. In the case of lung carcinogenesis, an understanding of the earliest phases has been further hampered by uncertainty about the precursor lesions for peripheral airway cell carcinomas. Although the sequence of premalignant changes in the bronchial epithelium from hyperplasia to metaplasia to dysplasia to carcinoma in situ and finally to invasive cancer has been well established for squamous cell carcinoma, this sequence is not nearly as well understood for adenocarcinomas, which have recently become the more common histologic variety of non-small-cell lung cancer. The precursor lesions for small-cell carcinoma are even less well understood. As a result, the temporal ordering of genetic lesions during lung carcinogenesis has been more problematic than in the case of colorectal carcinogenesis. Nevertheless, assignment of abnormalities into "early" (occurring at carcinoma in situ or earlier lesions) vs "late" (occurring after invasive cancer is identified) categories can still be achieved.

A second concept that is of particular importance to lung cancer biology is field cancerization, originally proposed by Slaughter et al. to describe oral carcinogenesis. Exposure of the entire aerodigestive tract to a carcinogenic insult (eg, tobacco) results in changes throughout this field yielding multiple independent foci of preneoplastic lesions that progress at different rates to form multiple primary cancers. This is reflected in the 4% to 7% annual incidence of second primary tumors in patients with primary carcinoma of the head and neck and lung. Biologic support for field cancerization can be found in several studies of multiple lesions in the aerodigestive tract. In one recent study of mutations of the p53 tumor suppressor gene in 31 patients with head and neck cancers and related secondary primary tumors, mutations occurred in only one primary tumor in 16 patients, while mutations in different exons of p53 occurred in four out of five patients with p53 mutations in more than one primary tumor. In one patient, the same exon of p53 was mutated in both primary tumors, but the specific codon was different in each case. Such discordant mutations provide evidence for the independent origin of these tumors. Further evidence for field cancerization can be found in the work of Sozzi et al. who examined multiple neoplastic and preneoplastic lesions of five patients with multiple lung cancers for abnormalities in p53, K-ras, and chromosome 3p. The results of these studies showed different patterns of mutations in histologically distinct lesions from the same patients, again confirming the independent origin of these multiple lesions.

The implications of field cancerization are not only that the presence of multiple concurrent preneoplastic foci makes the surrounding lung a rich source of precursor lesions to study for genetic abnormalities, but also that local therapies aimed at excising an established invasive cancer are insufficient to prevent future disease. This concept has led to a series of chemoprevention trials using the development of second primary tumors as an endpoint. Since the discipline of chemoprevention is predicated on the idea of prevention of tumor progression in the exposed epithelium, the natural corollary is to focus on the early carcinogenic events occurring in that epithelium both as targets for intervention and intermediate markers for these trials.
Molecular Biology of Early Lung Carcinogenesis and Applications to Intermediate Markers

It is useful to define the characteristics of an ideal intermediate endpoint marker before discussing the applications of molecular biology to such markers. Potential intermediate markers fall into several broad categories (Table 1) and should possess specific properties: (1) differential expression between normal and premalignant epithelium, (2) a low rate of spontaneous reversion, (3) a high association with the eventual development of cancer, (4) reduction or disappearance of the marker indicating control of the disease, (5) modulation by chemopreventive agents, and (6) detection in easily accessible small tissue fragments to permit serial studies.20,23

<table>
<thead>
<tr>
<th>Intermediate Markers</th>
<th>Characteristics</th>
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<tr>
<td>Histology</td>
<td>Cytologic changes, increased mitotic activity, increased cellularity, increased cell density</td>
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<tr>
<td>Differentiation markers</td>
<td>Keratin expression, loss of terminal differentiation markers</td>
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<tr>
<td>Epithelial markers</td>
<td>ONCO1, K1-31</td>
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<tr>
<td>Oncogenes</td>
<td>p53, c-erbB-2, her2neu</td>
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<tr>
<td>Tumor suppressor genes</td>
<td>p16, p15, Rb</td>
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<tr>
<td>Cell cycle control genes</td>
<td>Cyclin D1, p53</td>
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<td>Growth factor receptors</td>
<td>Epidermal growth factor receptor</td>
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Historically, histopathology has been the standard for intermediate markers. In the case of colorectal carcinogenesis where the progression of adenomas to carcinomas is well established, development of adenomas has been used as an endpoint in assessing efficacy of potential chemopreventive agents such as aspirin.24 In lung carcinogenesis, however, attempts to use squamous metaplasia (characteristic of an early phase of carcinogenesis) as an intermediate endpoint have been less successful. In a study by Lee et al,25 reversion of metaplasia was seen equally between isotretinoin- and placebo-treated patients, with the greatest correlation to cessation of smoking. This suggests that squamous metaplasia is too early a change to be useful as an intermediate endpoint, and perhaps a later change such as dysplasia may be more specific. The need to prospectively validate intermediate markers, using development of cancer as the gold standard, applies to histologic surrogates as well as molecular surrogates.

Recent technical advances have turned the focus from histology to the potential use of molecular markers as intermediate endpoints. While a variety of genes demonstrate dysregulated expression in invasive small-cell or non-small-cell lung cancers (Table 2), their usefulness as intermediate markers in chemoprevention trials depends on the point during the course of carcinogenesis at which this dysregulation occurs. Ideally, intervention to arrest tumor progression would occur at a preinvasive phase of carcinogenesis (carcinoma in situ or earlier). The preferred markers for this process should be expressed during these preinvasive phases, but after the commitment to subsequent malignant development. Marker expression at a reversible phase (ie, bronchial hyperplasias seen with acute injury as well as carcinogenesis) would not provide sufficient specificity. As noted previously, bronchial metaplasia is an example of a marker that is reversible and therefore uninformative regarding the effect of an intervention agent.25 Improved understanding of the early events in lung carcinogenesis will be required to correlate morphologic features with potential molecular markers to validate marker expression and to demonstrate prospective correlation with development of cancer.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Histology</th>
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<tr>
<td>p53</td>
<td>SQC, SQC, LC</td>
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<tr>
<td>Retinoblastoma</td>
<td>LC, SQC</td>
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<tr>
<td>P16</td>
<td>SQC</td>
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<tr>
<td>SQC</td>
<td>Small cell lung cancer</td>
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<td>NSCLC</td>
<td>non-small-cell lung cancer</td>
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<tr>
<td>p16</td>
<td>Lung carcinoma</td>
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To date, a number of studies have begun to examine the expression of oncoproteins and tumor suppressor genes that are commonly dysregulated in lung cancer in preneoplastic lesions of the lung. Oncogenes are genes whose overexpression leads to dysregulated growth and carcinogenesis, while tumor suppressor genes are genes whose absent expression contributes to the neoplastic phenotype. The potentially causal relationship between these genes and the development of cancer makes them attractive targets for intermediate endpoint analysis, although their ultimate use remains to be proven.

The p53 tumor suppressor gene, one of the most frequently mutated genes in cancer, is a transcription factor that blocks entry of the cell into S phase and thereby prevents proliferation.26 The p53 gene has been found to be abnormal in approximately one third of mild to moderate bronchial dysplasias, with increasing frequency to 60% of severe dysplasias and carcinomas in situ.21,27,28 A recent retrospective, nested-case control study29 of patients with chronic obstructive pulmonary disease demonstrated the presence of anti-p53 serum antibodies in 23% of patients with cancer (lung, breast, and prostate) at five to 11 months prior to the diagnosis of cancer. Although this study was small and the percentage of patients with anti-p53 antibodies was low, the ability to precede the histologic diagnosis of cancer suggests that molecular targets may indeed be useful as intermediate markers. The p53 gene is an appealing target for intermediate endpoint analysis based on its central role in the development of cancer, its high frequency in lung as well as other cancers, its increasing expression during early bronchial carcinogenesis, and the ease of analysis by immunohistochemistry.

A number of other tumor suppressor genes, such as the retinoblastoma gene (Rb), have been found to be absent or mutated in lung cancer, although their expression in early lung carcinogenesis has not been as well characterized as that of p53. Deletions of portions of chromosome 3p, however, have been found to be consistent findings in almost 100% of small-cell lung cancers and squamous cell lung cancers, with frequent losses in other histologic subtypes of non-small-cell lung cancer as well.30 Chung et al31 studied the occurrence of p53 and 3p alterations in premalignant bronchial lesions in 17 patients by immunohistochemistry, loss of heterozygosity (LOH) analysis, and sequencing. They showed that allele loss on chromosome 3 precedes damage to the p53 gene, and the pattern of alterations suggested that damage to chromosome 3 may be progressive. Other studies of p53 loss in premalignant lesions also suggest that p53 loss is a very early and frequent event during carcinogenesis, occurring in 76% of hyperplasias. The occurrence of discordant allelic loss of 3p (ie, different alleles are lost in the tumor and the premalignant...
lesion) provide further proof for the concept of field carcinogenesis. The pattern of allelic losses at 3p suggests that a number of tumor suppressor genes may reside at this locus. The von Hippel-Lindau gene, located at 3p25, is frequently mutated or methylated in renal cancers but rarely in lung tumors. The recent identification of the FHIT gene at 3p14.2 as a candidate tumor suppressor gene and the subsequent demonstration of abnormalities in RNA transcripts in 80% of small-cell lung tumors and in 40% of non-small-cell lung cancers (with 76% of tumors demonstrating loss of FHIT alleles) suggests a role for this gene in lung carcinogenesis. The FHIT protein has high homology to the yeast enzyme diadenosine 5′,5′-diphosphate (ApA) asymmetrical hydrolase, which cleaves the ApA substrate into ATP and AMP. High levels of ApA have been detected at the G1-S boundary and have been proposed to stimulate DNA polymerase activity. Thus, it has been suggested that the loss of FHIT function could result in the constitutive accumulation of ApA and the stimulation of DNA synthesis and proliferation. While this mechanism is consistent with the finding of deletions of 3p at the early (i.e., hyperplasia) stages of carcinogenesis, whether FHIT is indeed the target gene being deleted remains to be proven through analysis of preneoplastic lesions. Its use as an intermediate marker is even more questionable, since alterations in expression at the reversible hyperplasia stage may be too early for clinical utility.

Deletions of chromosome 9p are also very frequent in invasive lung cancer as well as a variety of other tumors, and recent studies have identified the p16 tumor suppressor gene at the 9p21 locus. The p16 gene is an inhibitor of activated cyclin-D-Cdk4 complexes, preventing cell cycle progression. Its absence, usually by homozygous deletion or transcriptional silencing due to methylation, can lead to deregulated growth. Alterations in p16 have been well documented in non-small-cell lung cancer (cell lines as well as primary tumors), although abnormalities in preneoplastic lesions have not been reported yet. However, losses at the chromosomal region 9p21 have been found to occur as early as the hyperplasia stage during lung carcinogenesis, and a recent study of oral leukoplasia (the premalignant lesion for oral cancer) also found losses at 9p21 in approximately one third of patients. In this latter study, losses at both chromosomes 3p14 and 9p21 were examined, and of the 19 patients with losses at one or both loci, 37% subsequently developed cancer, while only one of 18 (6%) patients without chromosomal loss went on to develop cancer.

The data with p16 underscore the potential importance of cell cycle regulation in the development of cancer. Data also exist implicating cyclin D1 overexpression in non-small-cell lung cancer pathogenesis, with cyclin D1 clearly being a target of p16 action. Alternative mechanisms for abrogating a particular pathway during carcinogenesis, such as loss of an inhibitor (ie, p16) or gain of an inducer (ie, cyclin D1), point to the central nature of these pathways in the control of cell fate. Focus on uncovering the role of these pathways during early lung carcinogenesis may reveal important targets for intermediate endpoint analysis.

Among the dominant oncogenes that have been well characterized in various phases of lung carcinogenesis, K-ras is perhaps the most extensively studied. The ras family of proteins is involved in signal transduction, and point mutations in codons 12, 13, and 61 of K-ras lead to constitutive activation, perpetuating a growth stimulatory signal. K-ras mutations have been documented in 30% to 50% of lung adenocarcinomas, depending on the sensitivity of the assays used, and have been correlated with poor prognosis. Recent data have demonstrated K-ras mutations in atypical alveolar hyperplasias (a potential preneoplastic lesion for adenocarcinoma of the lung), although the exact timing of ras mutations during lung carcinogenesis is not well established. However, in a retrospective study, Mao et al were able to detect K-ras mutations in sputum of patients who subsequently developed K-ras positive tumors up to four months prior to diagnosis. In the same study, p53 mutations were detected in one of two patients who developed a p53-positive tumor 13 months later. Such results suggest that K-ras mutations occur early, but the specificity for cancer remains to be determined. The capacity to detect mutations in specimens such as sputum, obtained in a noninvasive fashion, is important for clinical application of intermediate endpoints.

Another dominant oncogene whose expression has been examined in early lung carcinogenesis is c-jun, a key component of the AP-1 transcription factor that mediates tumor promotion. The c-jun oncogene was found to be expressed by immunohistochemistry in 88% of bronchial atypical lesions and in 40% of alveolar atypical lesions, but in only 31% of invasive tumors. Whether this early expression of c-jun is specific for cancer, however, can be ascertained only by examining lung lesions associated with nonmalignant lung pathology.

Alterations in growth factor signalling pathways also have been well documented in lung carcinogenesis, and targeting of such pathways for intervention and intermediate endpoint analysis has been reviewed. The epidermal growth factor receptor (EGFr), in particular, has been found to have increased expression in a variety of lung carcinomas, and several studies have shown increased expression in preneoplastic lesions such as squamous metaplasia. Independent use of EGFr expression as an intermediate endpoint for chemoprevention, however, is not yet warranted since decreased EGFr expression was dependent on reversal of bronchial metaplasia and not independently associated with 13-cis-retinoic acid (13cRA) treatment in a recent study by Kurie et al.

Several other markers of carcinogenesis have been examined for their potential use as intermediate endpoints or prognostic markers. Microsatellites are highly polymorphic tandem DNA sequences showing alterations in the number of these repeats in a variety of hereditary and nonhereditary cancers. Microsatellite instability has been detected in approximately one third of tumors and histologically normal bronchi from patients undergoing resection for lung cancer. This suggests that microsatellite instability is a very early lesion and may be a marker for increased cancer risk. Similarly, telomerase, an enzyme that maintains chromosomal ends to prevent the progressive chromosomal shortening that is inherent to DNA replication, has been found to be reactivated in 80% of primary lung cancers and in 4.4% of normal adjacent controls. Telomerase activity appears to be present in the vast majority of tumors of all types, and considerable enthusiasm has been generated in the scientific community regarding its potential to be a specific marker for cancer. Examination of microdissected bronchial atypical lesions showed telomerase activity ranging from 25% in hyperplasias to 100% of carcinomas in situ. Noting that telomerase dysregulation is a very early event during lung carcinogenesis. The potential for such early events to be useful intermediate endpoint markers awaits clinical trials.

Perhaps the most promising intermediate endpoint marker to date is retinoic acid receptor-β (RAR-β). As will be discussed below, retinoids have been shown to be effective chemopreventive agents for second primary tumors in lung and head and neck carcinogenesis. Retinoids have important effects on the growth and differentiation of bronchial epithelial cells and mediate these effects through nuclear receptors. Xu et al have shown that RAR-b expression is decreased in dysplastic (56%) and overtly malignant (35%) lesions in head and neck carcinogenesis. Furthermore, Lotan et al showed that RAR-b expression is restored by isotretonin treatment and correlates with clinical response. Thus, RAR-b fulfills more of the characteristics of intermediate endpoint markers outlined above than any other potential marker. Prospective validation in larger clinical trials is now mandatory.

Chemoprevention Clinical Trials

As previously noted, bronchial metaplasia has been examined as a potential intermediate endpoint in the study of chemoprevention of lung cancer. An initial uncontrolled trial by Misset et al evaluating 25 mg/d of etretinate for six months in heavy smokers reported a reduction in the mean bronchial metaplasia index from 35% before treatment to 27% following treatment. Arnold et al evaluated reversal of metaplasia in sputum samples after treatment with etretinate for six months and found no significant difference in response between the etretinate and the placebo groups. A four-year placebo-controlled study of 13cRA against bronchoscopically
documented bronchial metaplasia found no significant retinoid activity with reduction of metaplasia in 54% of patients taking 13cRA and in 59% of placebo subjects. In this trial, the greatest reduction in bronchial metaplasia was associated with smoking cessation. Other randomized, placebo-controlled trials have incorporated metaplasia as determined from sputum samples as a potential intermediate endpoint. Heimbucher et al\(^9\) conducted a placebo-controlled, randomized trial of smokers with metaplasia determined by sputum cytology with folic acid and vitamin B12 for four months. This trial reported a significant improvement in dysplasia in the treatment group ($P=0.02$).

A trial currently is underway to study various premalignant markers in high-risk patients following smoking cessation. The validation of these biomarkers as early markers for lung cancer is still ongoing. The identification of more precise intermediate endpoints will facilitate the development of chemopreventive agents to target specific preneoplastic molecular events.

Retinoid treatment in the prevention of second primary tumors, in contrast to studies of bronchial metaplasia, has shown promising results. Pastorino and colleagues\(^60\) randomized 307 patients who had complete resections of stage I non-small-cell lung cancer to either 300,000 IU of retinyl palmitate or no treatment for 12 months. With a median observation of 46 months, time to development of second primary tumors in the aerodigestive tract was significantly longer in the treatment arm ($P=0.045$).

Two active chemoprevention trials with the endpoint of second primary lung cancer are currently ongoing. The EUROSCAN Trial is a large phase III European trial involving 13 countries and 40 cancer centers. This trial is using a two-by-two factorial design to test retinyl palmitate (300,000 IU/d in year 1 and 150,000 IU/d in year 2) and N-acetylcysteine (600 mg/d for two years) in patients previously treated for early-stage squamous carcinoma of the larynx, oral cavity, or early-stage non-small-cell lung cancer. The US Phase III Intergroup Study of low-dose 13cRA (30 mg/d) began accruing patients with resected stage I non-small-cell lung cancer in December 1992. The target sample size for this study is 1,260, and accrual is expected to be completed in 1997.

The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study was a primary chemoprevention trial that enrolled more than 29,000 male smokers between the ages of 50 to 69 years in southwest Finland.\(^61\) This randomized, double-blind, placebo-controlled trial used a two-by-two factorial design to assign treatment with 50 mg/d of alpha-tocopherol, 20 mg/d of beta-carotene, both, or placebo with a follow-up of five to eight years. The beta-carotene arm showed a statistically significant 18% increased risk of lung cancer, with no change in the alpha-tocopherol group.

The Beta-Carotene and Retinol Efficacy Trial (CARET) was a six-center phase III randomized, double-blind, placebo-controlled trial designed to test the effect of 25,000 IU of vitamin A and 30 mg of beta-carotene per day in preventing lung cancer in 14,420 heavy smokers as well as 4,010 workers exposed to asbestos. Interim analysis of the CARET trial was conducted in January 1996 and demonstrated a 28% increase in lung cancer incidence in the beta-carotene arm.\(^62\) The surprising finding of an increased lung cancer risk in these two trials suggests that a dietary supplement cannot provide all the elements in yellow and green leafy vegetables that confer protective benefits in epidemiologic studies. Furthermore, there may be a harmful interaction in smokers taking the beta-carotene supplement contributing to the increased risk.

The Physicians’ Health Study was started in 1982 and is comprised of male physicians in the United States testing beta-carotene and aspirin in the prevention of cancer and cardiovascular disease.\(^53\) Analysis of this study of predominantly nonsmokers shows no difference in lung cancer risk associated with beta-carotene use with 12 years of follow-up. Several large ongoing randomized trials are evaluating agents in the high-risk heavy-smoker population. Table 3 (see hard copy) shows the current large-scale trials being conducted for the chemoprevention of lung cancer.

The discovery of a successful chemopreventive agent for lung cancer would also play an important role in survivors of small-cell lung cancer. Long-term survivors of small-cell lung cancer have an extremely high rate of development of second primary tumors, and in two recent studies, second primary non-small-cell lung cancer was the primary cause of cancer death four years after primary therapy.\(^19,64,65\)

Conclusions

The promise of preventing neoplastic disease must now be followed with tools to translate the promise into a clinical reality within a reasonable time frame. As our understanding of the molecular mechanisms driving lung carcinogenesis deepens, numerous potential molecular targets for intervention as well as detection of early disease will become available. The careful validation of these molecular tools will require an increased understanding of the early events in pulmonary carcinogenesis followed by prospective clinical trials. While current research is beginning to focus on the expression of molecular markers during the early stages of carcinogenesis, few studies thus far have explored the expression of these cancer related genetic changes in nonneoplastic pulmonary pathologies. Such specificity issues will become more important as potential intermediate endpoint markers enter into clinical trials. The simultaneous development of early intervention strategies and intermediate endpoints will be crucial to reducing mortality from lung cancer. The ability to target specific molecular events in carcinogenesis with chemopreventive agents could lead to the reversal of this process and ultimately to decreased lung cancer incidence.

References

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