Lung cancer remains the leading cause of cancer death in the United States and is one of the world’s leading causes of preventable death. Technologic advances have brought new modalities that may be useful for the early detection of lung cancer. However, because of the large number of persons at increased risk for lung cancer, screening is a formidable task. There are several risk factors that can be identified, including potential susceptibility factors, which may aid in pinpointing individuals who need to participate in regular screening programs. Aside from recognized environmental exposures including cigarette smoking, there are a number of genetic and metabolic susceptibility factors that have been examined. These include polymorphisms in the cytochrome p450 enzymes and the metabolizing capability of glutathione s-transferase or acetylation. Additionally, defects in DNA repair and in bleomycin sensitivity assays may also aid in identifying individuals who are at an increased risk for lung cancer.

Additional work has been done in the area of characterizing the molecular alterations in the bronchial epithelium in high-risk smokers. This manuscript addresses only selected molecular alterations that have been examined in preneoplastic bronchial epithelium. In addition to mutations in the k-ras oncogene and the p53 gene, which are frequently seen in malignancy, alterations in the p16 gene, microsatellite instability and loss of heterozygocity are also promising potential markers of preneoplasia. The hnRNP A2/B1 gene also shows some promising increased expression in preneoplasia.

Lung cancer prevention has made some strides. A number of trials with molecular and morphologic intermediate endpoints have been conducted and have suggested that some of the molecular alterations and morphologic alterations are reversible. However, the rate of spontaneous regression of these lesions is, as yet, uncharacterized. Two recent large studies, the beta-carotene and retinol efficacy trial (CARET) trial conducted in the United States and the Alpha-Tocopherol Beta Carotene (ATBC) trial conducted in Finland, both demonstrated an unexpected increased risk for lung cancer associated with beta-carotene supplementation. The EUROSCAN trial evaluation of vitamin A and N-acetylcysteine also showed no benefit to supplementation in reducing risk for lung cancer. Results from the Intergroup study of 13-cis-retinoic acid showed no benefit to supplementation in reducing risk for lung cancer. Hopefully, the combination of identifying markers of increased risk among the numerous current and former smokers will identify high-risk populations to participate in future trials of promising agents that may lead to reduction in incidence and mortality of the leading cause of cancer death.

Environmental tobacco smoke continues to be a source of lung cancer risk as demonstrated by studies showing that non-smoking women whose husbands smoked cigarettes were at higher risk of lung cancer than those whose husbands were non-smokers [4,5,6]. Fortunately changes in the workplace and many other public places have decreased exposure to environmental tobacco smoke.
Environmental exposures
There are a number of sources of exposure to occupational hazards that increase lung cancer risk. Radon was identified as a cause of lung cancer in underground miners [7]. Other occupational causes of lung cancer include polycyclic aromatic hydrocarbons, nickel, chrome, arsenic, asbestos, and chloromethyl ethers [8]. Additionally, coke oven workers who were exposed to compounds containing benz[a]pyrene are at an increased risk for lung cancer [9,10]. Radiation also can increase lung cancer risk. Studies of patients who received radiation therapy for Hodgkin’s disease demonstrate that particularly in patients who continue to smoke cigarettes there is an increased risk for developing subsequent lung cancers in the radiation field. There also have been similar studies examining subsequent lung cancer risks in women treated with breast conserving therapy followed by radiation therapy.

Acquired lung disease
Studies of individuals with chronic obstructive pulmonary disease as well as those with pneumoconiosis have examined the relationship of these problems to subsequent lung cancer risk. Tockman [11] demonstrated that the degree of airflow obstruction was a predictive factor for lung cancer risk. Examination of the association between pneumoconiosis has been less clear.

Susceptibility factors
Given the observation that less than twenty percent of cigarette smokers will eventually develop lung cancer, a variety of susceptibility modifiers have been postulated. Familial aggregation of lung cancer has been examined in case control studies [12,13,14]. Although these studies tended to adjust for smoking, it is clearly demonstrated that smoking aggregates in families and acts as a major confounder in this evaluation. Sellers conducted a segregation analysis suggesting that lung cancer was inherited in an autosomal-codominant pattern in families in Louisiana [15]. Another large study of 15,924 male twin pairs in the United States did not support a genetic basis for lung cancer susceptibility [16].

Aside from familial inheritance patterns, there may be metabolic susceptibility related to variations in metabolizing tobacco-associated carcinogens. Differential rates of activation or inactivation of carcinogens related to polymorphisms of metabolism controlled by either cytochrome p450 genes, glutathione s-transferase, or acetylation have all been postulated to play a role in lung cancer susceptibility. A number of studies of p450 enzymes including CYP1A1 and CYP2D6 have been conducted. Some, although not all, studies have reported increased lung cancer risk associated with certain polymorphisms. There is a clear ethnic variation in the findings of these studies; however, methodologic variation in studies most likely accounts for variations in results [17].

Spitz et al. [18] have examined a bleomycin sensitivity assay as a possible marker for lung cancer susceptibility and demonstrated an increased risk with increasing frequency of aberrations. They have studied other DNA repair capacity markers and also suggest that DNA repair capacity was lower in lung cancer cases than it was in controls.

The capability to use one of the many proposed assays to identify susceptible subgroups raises the potential to target those individuals identified as being at the highest risk of lung cancer for potential early detection intervention studies. The over 90 million current and former smokers at risk for lung cancer in the United States pose too large a group for mass screening or intervention. Identifying a subgroup with validated susceptibility markers could focus screening and intervention efforts on the very high-risk cohorts [19].

Molecular characterization of high-risk populations
In lung cancer the earliest changes occur in the bronchial epithelium. Changes in these cells detectable either by examining cells shed into sputum or obtained by bronchoscopy may provide a way to evaluate transformation occurring on the surface epithelium [20]. Recent advancements in molecular methodologies may provide a useful tool in the early detection of lung cancer and the identification of high-risk persons with preneoplastic bronchial dysplasia [21]. Work on tumor cell lines and lung tumor tissue has identified a variety of molecular markers and specific mutations that may be involved in lung cancer pathogenesis. These changes include amplification or overexpression of oncogenes, deletion of tumor suppressor genes, or loss of tumor suppressor gene expression [22]. Chromosome instability can be characterized by loss of heterozygosity or microsatellite instability. Loss of genes or gene function may alter cellular regulation and lead to abnormal proliferation.

Currently histology remains the first avenue for evaluation of the bronchial epithelium. Squamous metaplasia has not been demonstrated as an adequate intermediate marker of a preneoplastic lesion. Bronchial dysplasia is currently under evaluation for its usefulness as an intermediate marker [21]. Recent studies suggest that biopsy of bronchial dysplasia results in regression of the dysplasia without any additional intervention (Gazdar; personal communication).

Molecular evaluation is also currently being examined to determine the relationship between alterations at the
genetic level and their association with eventual development of lung cancer.

**Ras oncogenes**

Mutations in the *k-ras* oncogene are found in thirty to eighty percent of non–small cell lung cancers [23,24,25]. *Ras* mutations have been identified in sputum and bronchial lavage samples from persons not yet known to have cancer [24,25,26,27]. Therefore, development of the molecular assays for *ras* mutations may provide a reasonable screening test in appropriate individuals.

**p53**

The *p53* gene is the most frequently mutated gene in lung malignancy. Mutations in the *p53* gene can be evaluated on the basis of excess accumulation of *p53* protein in the nucleus or by using molecular assays to evaluate for specific mutations. Ahrendt [20] demonstrated that *p53* mutations could be detected in the bronchial lavage fluid of 39% of non–small cell lung cancer patients with identifiable mutations. Mao et al. [27] also identified specific *p53* mutations in archival sputum samples from patients who subsequently developed lung cancer. These investigators used specific molecular techniques that are not adaptable to a screening situation; however, this study does demonstrate that specific molecular alterations can be detected in noninvasive samples such as sputum.

**p16/INK4**

The *p16* gene normally inhibits the CDK4/cyclinD1 kinase activity involved in phosphorylation of the retinoblastoma protein. *p16* gene mutations are seen in approximately 70% of non–small cell lung cancer. The loss of *p16* expression allows the CDK4/cyclinD1 kinase activity to proceed unchecked and allows the unregulated progression of the cell cycle even if DNA damage has occurred. Belinsky et al. [28,29] have used methylation-specific polymerase chain reaction to show a correlation between the increased hypermethylation of the *p16* gene and a more severe grade of lung cancer.

**Microsatellite instability**

Repetitive sequences of DNA that contain either dimer, trimer, or tetramer repeat sequences are scattered throughout genomic DNA. When a mutation is present in the enzymes needed to repair damaged DNA, the number of repeats at a genomic site may expand or contract and create microsatellite instability. In colon cancer this has been demonstrated to result in replication error–prone repair. In non–small cell lung cancer 9p21-22 and 3p loci have been demonstrated to exhibit microsatellite instability [30,31,32]. It is possible that these types of changes could be identified on bronchial epithelium in individuals with preneoplastic changes. Further evaluation of this is currently underway.

**Loss of heterozygocity**

Loss of heterozygocity determines whether one, both, or neither allele of the gene are present at a specific locus. Loss usually suggests that a deletion has occurred. In current and former smokers loss of heterozygocity is seen in chromosomes 3p and 9p and less frequently on 3q, 17p, and 13q [33]. Metaplasia and dysplasia show partial loss of heterozygocity and carcinoma shows a complete loss of heterozygocity [34].

**hnRNP A2/B1**

The *hnRNP A2/B1* gene was identified following demonstration that a monoclonal antibody 703D4 showed increased expression an average of 2 years prior to the detection of lung cancer in a project examining archival sputum specimens from the John Hopkins Lung Project [35]. Additional evaluations of the usefulness of staining with this monoclonal antibody are being examined in patients with resected stage I non–small cell lung cancer and a cohort of Chinese tin miners. Others have suggested *hnRNP B1* may be more specific for lung cancer [36].

**Implications**

Recent advances in molecular technology result in an ability to characterize individuals for potential genetic susceptibility markers as well as the presence of molecular alterations on epithelial surfaces. As these markers are further characterized and validated it may become possible to identify a cohort of extremely high-risk individuals to target for early detection and intervention strategies. It will be necessary to validate the markers and to determine whether early detection strategies can improve lung cancer mortality rates and also to determine whether chemointervention strategies can reduce lung cancer incidence. The continued evolution of new methodology will increase the potential for evaluating molecular alterations on the cellular surface. The human genome project and the development of microarray techniques will enable the evaluation of tens of thousands of genes that may be regulated under different conditions. The ability to analyze gene expression and identify mutations in larger genes more rapidly than previously possible will allow characterization of these molecular changes in persons at risk for lung cancer and among those who eventually develop lung cancer. In the future, patients expressing certain genetic alterations may be targeted for specific interventions, and, in addition, we may find that these will be useful in recommending treatment for persons with cancers expressing these alterations.

Presently there are a host of molecular alterations that have been identified in lung tumors, only a few of which are mentioned in this review. The recent funding by the National Cancer Institute of an Early Detection
Network is the first step in validating markers in high-risk populations. True validation through following a cohort of high-risk individuals prospectively for the development of lung cancer will be the only accurate way to determine whether expression of certain molecular alterations or protein overexpression is predictive of lung cancer development. Once validated these markers can identify individuals for intervention and screening studies.

The recent explosion in molecular technologies affords us with an exciting opportunity to characterize individual susceptibility at a genetic level as well as the presence of early alterations in relevant tissues. Only through time and careful evaluation of the predictive role of these changes will we be able to intelligently use this information to identify at-risk individuals and define creative strategies for reducing the risk of lung cancer among these individuals.

**Lung cancer prevention**

Chemoprevention strategies in lung cancer followed successful experience gained in head and neck cancer research. Tobacco-related carcinogenesis affects an entire field in the upper aerodigestive tract. Patients with head and neck cancer who were definitively treated were randomized to receive either 13-cis-retinoic acid or placebo. Patients receiving the 13-cis-retinoic acid had a significant reduction in the incidence of second primary tumors and particularly second primary tumors in the aerodigestive tract [37,38].

A number of trials have been published attempting to examine the effect of chemointervention on pre-malignant lung cancer. An initial uncontrolled trial by Misset et al. [39] evaluated 25 mg a day of etretinate in heavy smokers for 6 months. They reported a reduction in the mean bronchial metaplasia index. Reversal of bronchial metaplasia was also examined in two randomized trials conducted in heavy smokers [40,41]. These studies showed no significant reduction in metaplasia index after etretinate or 13-cis-retinoic acid, but did show significant reduction in metaplasia index associated with smoking cessation [41].

Retinoid treatment in the prevention of second primary tumors has shown positive results. Pastorino et al. [42] conducted a randomized study of 307 patients with completely resected stage I non–small cell lung cancer evaluating 300,000 IU of retinyl palmitate versus no treatment for 12 months. Time to development of second primary tumors in the aerodigestive tract was significantly longer in the treatment arm after 46 months of observation ($P=0.045$). A large randomized primary chemoprevention trial conducted in over 29,000 male smokers between the ages of 50 and 69 in Finland examined a two by two factorial design administering alpha-tocopherol and beta-carotene (ATBC) [43]. Patients receiving beta-carotene had a statistically significant 18% increased risk of lung cancer with no change in the alpha-tocopherol group.

The CARET conducted in the United States was a phase III, randomized, double-blind, placebo-controlled trial of 25,000 IU Vitamin A and 30 mg beta-carotene daily in 14,420 smokers and over 4,000 asbestos-exposed workers. Interim analysis of this trial was conducted in January 1996 and demonstrated a 28% increase in lung cancer incidence in the beta-carotene arm [44]. The Physicians’ Health Study was composed of male physicians in the United States evaluating beta-carotene and aspirin in the prevention of cancer and cardiovascular disease [45]. Analysis of that cohort of predominately non-smokers has demonstrated no difference in lung cancer risk associated with beta-carotene use with 12 years of follow-up. Nevertheless, the surprising increased lung cancer risk in the ATBC and CARET studies suggests that a dietary supplement cannot provide all the elements in yellow and green leafy vegetables, which have been confirmed, in epidemiologic studies, to provide protective benefits. There is also a concern of potential harmful interactions in smokers taking the beta-carotene supplementation resulting in the increased risk.

The EUROCARE trial was reported at the 1999 American Society of Clinical Oncology meeting by Van Zandwijk [46]. This randomized clinical trial evaluated Vitamin A and N-acetylcysteine in a two-by-two factorial design in 2,592 patients following treatment with curative intent for early-stage head and neck cancer or lung cancer. Seventy-seven percent of the participants completed the prescribed 2-year intervention following a median follow-up of 49 months. There was no difference in time to recurrence, second primary, or death with either N-acetylcysteine or retinyl palmitate versus the placebos. There were no survival differences with either the N-acetylcysteine or the retinyl palmitate [46].

The intergroup study comparing 13-cis-retinoic acid with placebo in surgically resected, stage I, non–small cell lung cancer patients has completed accrual and results of that trial are pending. A successor intergroup trial is planned comparing high selenium yeast with placebo yeast in the stage I, non–small cell lung cancer resected population with treatment planned for 4 years. It is anticipated that this trial will open to accrual in early 2000.

Fenretinide (4-hydroxyphenylretinamide [4-HPR]) was administered to a population of high-risk smokers who had a bronchial metaplasia index greater than 15 percent
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or bronchial dysplasia on bronchoscopic biopsy in a placebo-controlled clinical trial. Eighty-two individuals were randomized to 4-HPR versus placebo. Metaplasia index decreased in both groups and dysplasia did not change. In addition, analysis of the data also showed that 4-HPR had no effect on RAR-beta expression. The conclusions were that 4-HPR in this study had no effect on the reversal of squamous metaplasia, dysplasia, or RAR-beta expression in bronchial mucosa [47].

It is certainly disappointing that none of the randomized chemoprevention trials with an endpoint of lung cancer have proven to show an intervention effect. Further investigation may lead to identification of an effective chemopreventive for lung cancer. Such an agent would play an important role in reducing lung cancer incidence, which would ultimately reduce lung cancer mortality.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

* Of special interest
** Of outstanding interest

36 Presents early results of evaluation of hnrN 2B/A1 in 2 prospective studies of persons at high risk for lung cancer.
38 Reports hnrN 2B/A1 specifically stained in human lung cancers and cell lines.


