# Fifty Years of Tobacco Carcinogenesis Research: From Mechanisms to Early Detection and Prevention of Lung Cancer

Stephen S. Hecht[1] and Eva Szabo[2]

## Abstract

The recognition of the link between cigarette smoking and lung cancer in the 1964 Surgeon General's Report initiated definitive and comprehensive research on the identification of carcinogens in tobacco products and the relevant mechanisms of carcinogenesis. The resultant comprehensive data clearly illustrate established pathways of cancer induction involving carcinogen exposure, metabolic activation, DNA adduct formation, and consequent mutation of critical genes along with the exacerbating influences of inflammation, cocarcinogenesis, and tumor promotion. This mechanistic understanding has provided a framework for the regulation of tobacco products and for the development of relevant tobacco carcinogen and toxicant biomarkers that can be applied in cancer prevention. Simultaneously, the recognition of the link between smoking and lung cancer paved the way for two additional critical approaches to cancer prevention that are discussed here: detection of lung cancer at an early, curable stage, and chemoprevention of lung cancer. Recent successes in more precisely identifying at-risk populations and in decreasing lung cancer mortality with helical computed tomography screening are notable, and progress in chemoprevention continues, although challenges with respect to bringing these approaches to the general population exist. Collectively, research performed since the 1964 Report demonstrates unequivocally that the majority of deaths from lung cancer are preventable. *Cancer Prev Res; 7(1); 1–8. ©2014 AACR.*

The 1964 Surgeon General's Report linking cigarette smoking and lung cancer has had an enormous positive effect on public health in the United States. Beginning then, male smoking prevalence decreased from 51.1% to the current 21.6%, whereas prevalence in females diminished from 33.3% to 16.5% (1–3). In parallel, but about 25 years later, lung cancer mortality in men began to decrease from its maximum of 91 per 100,000 to 60 per 100,000 in 2010, whereas female rates have decreased from their maximum of 42 per 100,000 in 2002 to 38 per 100,000 in 2010 (Fig. 1;

**Authors' Affiliations:** [1]Masonic Cancer Center, University of Minnesota, Minneapolis, Minnesota; [2]Lung and Upper Aerodigestive Cancer Research Group, Division of Cancer Prevention, National Cancer Institute, NIH, Bethesda, Maryland

**Corresponding Authors:** Stephen S. Hecht, Masonic Cancer Center, University of Minnesota, 2231 6th Street Southeast, Room 2-148 CCRB, Minneapolis, MN 55455. Phone: 612-624-7604; Fax: 612-624-3869; E-mail: hecht002@umn.edu; and Eva Szabo, Lung and Upper Aerodigestive Cancer Research Group, Division of Cancer Prevention, National Cancer Institute, NIH, HHS, 9609 Medical Center Drive, Room 5E-102, Bethesda, MD 20892. Phone: 240-276-7011; Fax: 240-276-7848; E-mail: szaboe@mail.nih.gov

ref. 4). Other tobacco-related diseases continue to decrease. These facts clearly demonstrate the critical importance of tobacco control in disease prevention and illustrate beyond any reasonable doubt that the majority of lung cancer is preventable. There are diverse approaches to lung cancer prevention, ranging from basic science investigations of tobacco smoke carcinogens to behavioral interventions, early detection, chemoprevention, and policy. This perspective will focus on mechanisms of tobacco carcinogenesis, early detection of lung cancer, and chemoprevention. We will present some highlights of this research (Table 1) and discuss what needs to be accomplished to further advance lung cancer prevention. For thorough discussions of tobacco cessation, control, and policy issues, which are also clearly critical for lung cancer prevention, we refer the reader to recent summaries published by the American Association for Cancer Research and the American Society of Clinical Oncology (5, 6).

## Selected Highlights in Tobacco Carcinogenesis Research

There have been significant advances in the characterization of carcinogens in tobacco and tobacco smoke. Improvements in analytical chemistry, particularly in mass spectrometry, have facilitated characterization of multiple compounds in tobacco, which has the typical complexity of any agricultural product, and in tobacco
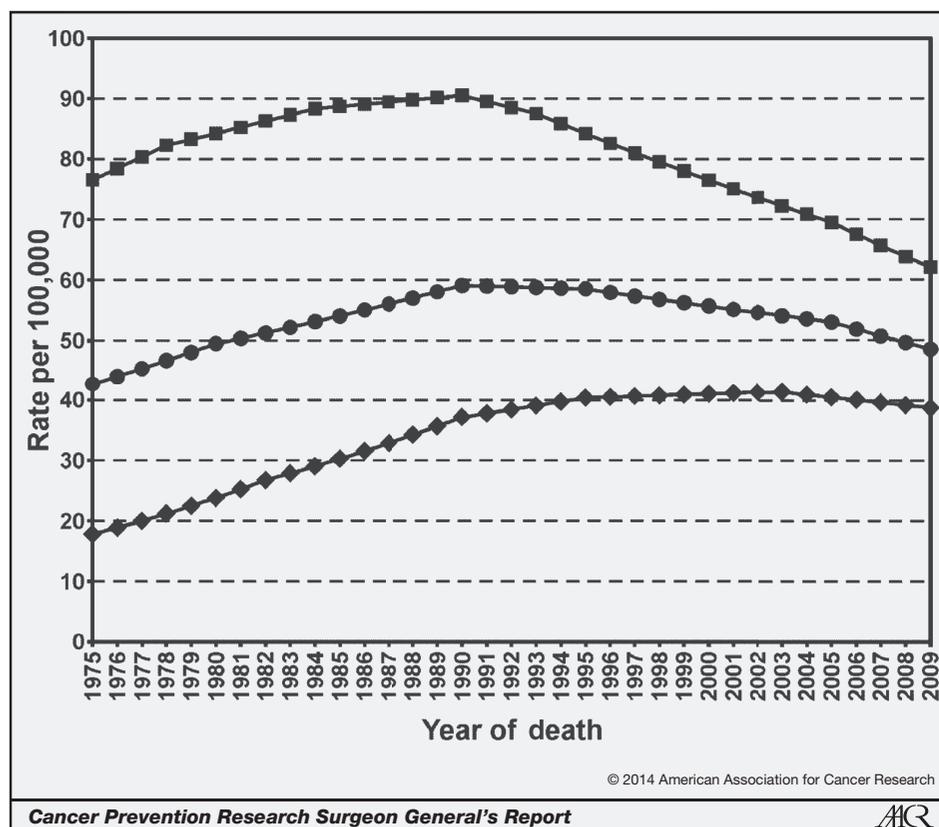
Figure 1. Age-adjusted total U.S. morality rates for lung and bronchus cancer, 1975–2009. Square, males; diamonds, females; and circles, both sexes. Source: SEER data (http://seer.cancer.gov).
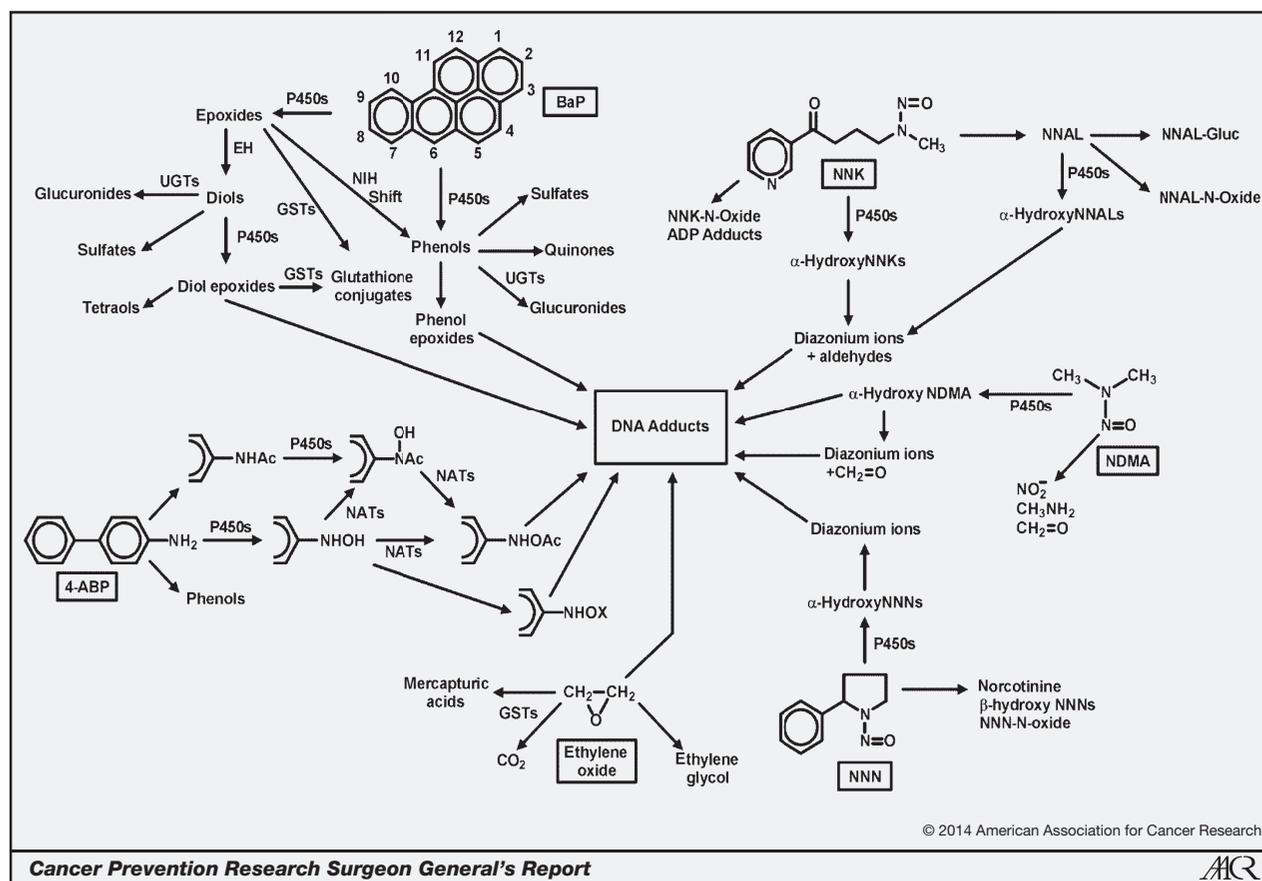
smoke, which is even more complex because the plant constituents are heated to at least 880°C during smoking. More than 8,000 compounds have been identified in tobacco and tobacco smoke (7). Among these are more

**Table 1.** Highlights of this article

- Tobacco control activities since 1964 have resulted in decreasing lung cancer mortality in the United States.
- Many carcinogens in tobacco products have been identified and the metabolic pathways leading to DNA adduct formation have been elucidated.
- Multiple DNA adducts are present in the lungs of smokers consistent with the thousands of mutations found in critical genes in lung cancer.
- Tobacco carcinogen and toxicant biomarkers provide an objective way to quantify dose, and possibly lung cancer risk, in smokers.
- Screening with helical CT has been shown to reduce lung cancer mortality
- Ongoing research is seeking to refine risk assessment models to focus screening resources to the highest risk populations
- Although no agents are approved for lung cancer prevention, promising agents and new clinical trials models are currently being tested

than 70 carcinogens classified by the International Agency for Research on Cancer as having sufficient evidence for carcinogenicity in either laboratory animals or humans (8, 9). These include polycyclic aromatic hydrocarbons, tobacco-specific nitrosamines, volatile nitrosamines, aromatic amines, aldehydes, volatile hydrocarbons such as benzene and 1,3-butadiene, miscellaneous other organic compounds, metals, and the radioelement $^{210}$Po among others, a carcinogenic brew which is far more diverse than imagined in 1964. Many of these carcinogens are arguably linked to the multiple cancers which occur in tobacco users, thus providing a starting point for rational prevention strategies. In this regard, it is critical to understand the structure of the enemy-strengths and weaknesses- to design suitable preventive approaches.

The concept of carcinogen metabolic activation to products that covalently bind to DNA, the bedrock of our understanding of chemical carcinogenesis, was just being developed by James and Elizabeth Miller at the time of the first Surgeon General's Report (10). Multiple well-known research groups investigated this process, particularly in the second half of the 20th century. These studies have convincingly demonstrated the ways in which virtually all organic carcinogens in cigarette smoke are metabolically activated (usually by cytochrome P450 enzymes) and detoxified [by P450s, uridine-5′-diphosphate-glucuronosyl transferases (UGT), glutathione $S$-transfrerases (GST), sulfatases, and others; ref. 11]. Figure 2 illustrates

**Figure 2.** Overview of the metabolism of six tobacco smoke carcinogens that produce DNA adducts that have been identified in lung tissue from smokers. The names of the six carcinogens are as follows, clockwise from the upper left-hand corner: BaP, NNK, NDMA, NNN, ethylene oxide, and 4-ABP. EH, epoxide hydrolase; NATs, N-acetyltransferases. BaP, benzo[a]pyrene; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NDMA, N-nitrosodimethylamine; NNN, N'-nitrosonornicotine; 4-ABP, 4-aminobiphenyl; NIH shift, rearrangement of an epoxide to a phenol; ADP, adenosine diphosphate; Ac, acetyl; in the 4-ABP scheme, X represents conjugates such as glucuronide or sulfate. Adapted from International Agency for Research on Cancer Monographs on the Evaluation of Carcinogenic Risks to Humans, Volume 83, Tobacco Smoke and Involuntary Smoking, p. 1006, and references therein.

some pathways by which six tobacco smoke carcinogens—benzo[a]pyrene (BaP), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), N'-nitrosonornicotine (NNN), N-nitrosodimethylamine (NDMA), ethylene oxide, and 4-aminobiphenyl (4-ABP)-–bind to DNA forming adducts that have been detected in the lung tissue of smokers (8). Multiple studies convincingly demonstrate the presence of DNA adducts in the lungs and other tissues of smokers, in quantities significantly higher than those found in nonsmokers, consistent with this presumed assault of metabolically activated carcinogens from cigarette smoke (12). Although we need to know more about the chemical structures of the common DNA adducts found in the lungs of smokers to focus our preventive approaches, their presence *en masse* is significant in itself. DNA adducts are critical in the carcinogenic process because they can cause miscoding during DNA replication. There are multiple DNA repair enzymes to excise adducts (13). Base excision repair, nucleotide excision repair, alkylguanine transferases, mismatch repair, and double-strand break repair, among

others, can return DNA to its normal unadducted state. If the repair systems are overwhelmed or inefficient, the result can be miscoding, often resulting in a $G \rightarrow T$ mutation, commonly found in the *TP53* and *KRAS* genes in lung tumors from smokers but significantly less frequently in nonsmokers (14).

Consistent with the presence of multiple DNA adducts in the lungs of the smokers, studies applying next-generation sequencing techniques to DNA isolated from lung tumors in smokers have demonstrated the presence of multiple mutations in critical genes. In one study, DNA isolated from 188 primary lung adenocarcinomas was sequenced. More than 1,000 mutations were identified in important cancer-related genes, including *TP53* and *KRAS* (15). Another study described mutations in a non–small cell lung cancer (NSCLC) from a person who had smoked 25 cigarettes per day for 15 years before removal of the tumor: more than 50,000 single-nucleotide variants were observed (16). A third study interrogated NSCLC and adjacent normal tissue for mutations and found an

average mutation frequency that was 10 times higher in smokers than in nonsmokers (17). These studies provide convincing evidence for the dire consequences resulting from exposure to, and metabolic activation of, multiple carcinogens in cigarette smoke.

Other investigations demonstrate the presence in cigarette smoke of free radicals and other agents that can induce oxidative damage, inflammatory substances such as acrolein and related compounds, cocarcinogens such as catechol, and tumor promoters that activate the NF-κB pathway (13, 18). Tumor promotion serves to exacerbate the mutational consequences of multiple DNA adducts by enhancing the proliferation of mutated cells that have been programmed for molecular pathways leading to cancer.

Our vastly increased understanding of the carcinogens and toxicants in cigarette smoke, along with studies on their metabolism in humans, has allowed the development of highly specific and quantitative biomarkers of carcinogen and toxicant uptake and metabolism in smokers and nonsmokers exposed to secondhand smoke (18). "Total nicotine equivalents," the sum of nicotine and five of its metabolites, has been particularly important, allowing determination of nicotine uptake in smokers. The plasma ratio of two of these metabolites—3′-hydroxycotinine: cotinine—reflects the ability of a smoker to metabolize nicotine and is a phenotypic marker of activity of cytochrome P450 2A6, the major nicotine-metabolizing enzyme (19). Smokers with high activity tend to smoke more because less nicotine remains in circulation (20). Related studies demonstrate the relationship to lung cancer of common variants in the *CRNA5–CHRNA3–CHRNB4* nicotinic acetylcholine receptor subunit gene cluster on chromosome 15q25 because of altered nicotine and carcinogen uptake (21–23). Another important tobacco-specific biomarker is 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and its glucuronides, metabolites of the powerful lung carcinogen NNK, which are found in the urine of all smokers, as well as in nonsmokers exposed to secondhand smoke (in lower concentrations; ref. 24). The detection of nicotine metabolites and NNAL in the urine of nonsmokers has played an important role in the development of the pervasive clean air regulations which we now enjoy but were unimaginable in 1964.

## Tobacco Carcinogenesis—What Needs to Be Done

The most straightforward method of preventing lung cancer is the elimination of tobacco smoking. Although considerable progress has been made, smoking prevalence in the United States has not changed very much in the past decade, and worldwide smokers number about 1.4 billion (2, 25). We need to continue those policies shown to be effective in tobacco control such as antitobacco advertising, taxation, and clean air regulations. Beyond that, how can mechanistic studies help in tobacco control?

Our deep understanding of carcinogens and toxicants in tobacco and tobacco smoke and their mechanisms of action, achieved during the past 50 years, provides the groundwork for product modification. This is now possible with passage in 2009 of the Family Smoking Prevention and Tobacco Control Act, which gives the U.S. Food and Drug Administration (FDA) power to regulate tobacco products. The FDA has developed a comprehensive list of "harmful and potentially harmful" constituents of tobacco products (26). It will be important to legally obligate the manufacturers of tobacco products to significantly decrease the concentrations of these carcinogens and toxicants in tobacco products, leading arguably to less dangerous products for those smokers who are addicted to nicotine and cannot break their habit. Nicotine replacement therapy is one clinically proven approach to improve abstinence from tobacco products (27) and emerging products such as "electronic nicotine delivery systems," commonly known as e-cigarettes, are potentially notable in this regard, but regulatory considerations are necessary.

One area of great potential importance is the identification of those smokers who are at high risk for lung cancer. Approximately 15% to 24% of lifetime smokers will get lung cancer (8). If these susceptible individuals could be identified at a relatively young age, intensive surveillance and tobacco cessation activities could be initiated, thereby decreasing their lung cancer risk. Presently, there are several statistical models available for identifying smokers highly susceptible to lung cancer, with varying degrees of reliability (28–32). None of these models includes tobacco carcinogen and toxicant biomarkers and all are retrospective in nature, including number of years of smoking as an important variable. It may be possible to use tobacco carcinogen and toxicant biomarkers to identify susceptible individuals at a young age when intervention would still be useful. Urinary metabolites such as total nicotine equivalents or NNAL, or polymorphisms in *CYP 2A6*, might fulfill this role by identifying smokers with higher carcinogen and toxicant exposure. DNA adducts or hemoglobin adducts of metabolically activated carcinogens might also be useful in this regard.

Although our understanding of individual carcinogens in tobacco smoke has advanced considerably, we are less able to describe the mechanistic effects of the whole mixture of smoke constituents as well as its complex subfractions. Such studies generally involve exposure of laboratory animals to smoke, an approach with many difficulties because laboratory animals will not voluntarily inhale tobacco smoke in the same way as humans (33). There are important unanswered mechanistic questions relevant to the whole mixture. These include the relative roles of individual constituents and the enhancing or inhibiting effects of multiple other constituents or subfractions. A clearer understanding of these aspects could perhaps improve the positive health impact of tobacco product regulation.

Mechanistic understanding can also suggest logical approaches to chemoprevention of lung cancer. Some agents being investigated such as naturally occurring indoles and

isothiocyanates can modify the metabolic activation and detoxification of tobacco smoke carcinogens in favorable ways (34). Some current aspects of lung cancer chemoprevention are discussed further below.

## Early Detection of Lung Cancer

Even as our understanding of the mechanisms of tobacco-induced carcinogenesis continues to evolve at the basic science level, significant progress has been made in clinical efforts to curb the progressive effects of tobacco carcinogens on the lung. To date, perhaps the greatest potential impact on the prevention of premature death from lung cancer comes from early detection research. Although not premised on mechanistic insights, the National Lung Screening Trial (NLST) served as an important milestone in reducing lung cancer mortality (35). Now, mechanistic insights may build upon that foundation, promising to further refine its methods and improve its impact. Focusing on a group at high risk for lung cancer as defined by demographic information (age and cumulative smoking exposure), the NLST definitively demonstrated that three annual screens with low-dose helical computed tomography (CT), as compared with similarly timed chest X-ray screening, resulted in a 20% decrease in lung cancer mortality in current smokers and former smokers who quit within the previous 15 years. Importantly, death from any cause was also significantly reduced in the CT arm by 6.7% (95% confidence interval, 1.2–13.6; $P = 0.02$), underscoring the outsized contribution of lung cancer to overall mortality in this group. These data contrast with prior large screening trials, such as the the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial, which showed no benefit with less sensitive screening methods such as chest X-rays (36).

However, the significant decrease in lung cancer death in the NLST was accompanied by a large number of false-positive results. Twenty-six percent of CT-screened participants eventually had at least one additional diagnostic procedure (additional imaging in most cases), whereas only 1.1% were diagnosed with lung cancer. These data point to the importance of identifying subgroups of smokers with substantially higher risk than targeted in the NLST. Furthermore, they also point to the need for identifying algorithms that incorporate the baseline CT findings in recommendations for subsequent follow-up. The research in this area is currently evolving rapidly. Kovalchik and colleagues showed that the majority of the benefit from CT screening occurred in the 60% of participants who were at the highest risk for lung cancer, as determined by factors such as age, body mass index, family history of lung cancer, pack-years of smoking, years since smoking cessation, and emphysema (37). A screening strategy focusing on this subgroup would identify 88% of the screening-prevented lung cancer deaths, while decreasing the false-positive rate. However, such a strategy would still require screening of large populations of eligible smokers, with its attendant health care costs and implications thereof.

Building on the advances in imaging technology, several groups have focused on incorporating demographic and baseline CT screening data to develop risk prediction models that could be used to recommend differing intensities of follow-up based on individual risk or to identify the highest risk individuals who would be eligible for chemoprevention intervention trials. Maisonneuve and colleagues in Italy and McWilliams and colleagues in Canada used data from multiple different CT screening programs to develop such risk prediction models (38, 39). The latter study, which added demographics to multiple variables associated with individual nodules [e.g., size, radiographic characteristics (nonsolid, part solid, solid nodules), lung location, and spiculation], was able to accurately estimate the probability that lung nodules detected at baseline CT are malignant, with an area under the receiver-operating characteristic (ROC) curve of more than 0.90.

Alternatively, research focusing on molecular biomarkers from a variety of biologic fluids or tissues also offers opportunities to complement or maybe even replace imaging in the early detection of lung cancer. In addition to the tobacco smoke carcinogen and toxicant biomarkers noted above, candidate markers with potential range from aberrantly methylated genes or microRNAs in sputum or blood to serum autoantibodies, plasma proteins, transcriptomic markers in bronchial brushings or serum, and volatile organic compounds in exhaled breath condensate (reviewed in 40, 41). As an example, a transcriptomic biomarker that was developed from the gene expression analysis of histologically normal bronchial brushings in patients with lung cancer showed 83% accuracy in diagnosing lung cancer when used alone, whereas sensitivity and negative predictive value both increased to 95% when combined with cytopathology (42). Subsequent work from the same group has shown that gene expression changes in the nasal and buccal epithelia reflect changes in the bronchial airways, thereby raising the possibility that these easily accessible surrogate tissues could aid in the early detection of lung cancer (43). Similarly, a novel optical technology (partial wave spectroscopic microscopy) detects nanoarchitectural changes associated with field carcinogenesis; early data suggest that application to cells from the buccal mucosa has potential to detect lung cancer, with an area under the ROC curve of more than 0.80 (44). Taken together, these data suggest novel avenues for exploration of biomarkers from surrogate tissues that can be exploited for early cancer detection.

## Chemoprevention of Lung Cancer

Complementary to efforts to identify lung cancer at an early curable stage are efforts to prevent the development of invasive disease in the first place. The rationale for lung cancer chemoprevention is based on the understanding of the development of lung cancer as a lengthy process that occurs over time in response to damage from tobacco carcinogen exposure, with the entire exposed epithelial

surface being subject to damage and, thus, "at-risk" (45, 46). Given that metastatic lung cancer is not curable, there is intuitive appeal to the concept of prevention. However, there are many challenges to the unequivocal demonstration of clinical efficacy. These include the difficulty in identifying adequately high-risk individuals to optimize the balance between the risk of and benefit from the intervention, the inherent challenges of showing preliminary efficacy in phase II trials in which one cannot directly assess the effect of interventions on cancer development because the participants do not yet have cancer, and, ultimately, the need for lengthy large phase III trials with cancer endpoints to show that a strategy works. Furthermore, the molecular heterogeneity of lung cancer, with identification of a number of different potential driver mutations, raises the possibility that different interventions may be necessary for different molecular subtypes of lung cancer (15, 17). Although no agents are approved for the prevention of lung cancer, recent progress in identifying potentially efficacious agents and very high-risk populations, as well as new study designs, provide a path for moving forward.

Potential agents to be used for cancer prevention can be identified via multiple means, including understanding of the molecular mechanisms of carcinogenesis, studies in laboratory animals, epidemiologic data, and secondary endpoints from clinical trials performed for other indications. Among the most intriguing recent leads is the analysis by Rothwell and colleagues, who performed a combined analysis of patient level data from multiple aspirin prevention studies and reported a 32% decrease in death from lung adenocarcinomas with aspirin use (47). The decrease in lung cancer deaths only became significant after 5 or more years of treatment, suggesting an effect on cancer incidence and perhaps the earlier stages of carcinogenesis, and was not dose dependent. Aspirin also reduced death from other cancers, such as colorectal and esophageal cancers. There is considerable appeal to a strategy that would be useful for the prevention of multiple chronic diseases and uses a drug that is as cheap and whose side-effect profile is as well understood as that of aspirin. Several phase II trials exploring the effects of aspirin on biomarkers of lung carcinogenesis should help to further define the role of aspirin in lung cancer prevention. Conceivably, applying the risk prediction model described by McWilliams and colleagues, as discussed above, could lead to an efficient way to perform a definitive phase III trial (39).

A second evolving area of investigation that could revolutionize the approach to studying cancer prevention and more efficiently identify potential effective agents concerns the phosphoinositide 3-kinase (PI3K) pathway and drugs that inhibit it. Recent data indicate that PI3K is frequently mutated in squamous cell carcinomas arising from tobacco-damaged epithelia, such as the lung and head and neck (48–50). Furthermore, activation of the PI3K pathway occurs early during lung carcinogenesis and a small phase I chemoprevention trial showed that intervention with a drug, myo-inositol, an effective lung tumor preventive agent in mice, leads to regression of bronchial premalignancy and concomitant inhibition of PI3K activation as judged by gene expression analysis (50). These results suggest that PI3K activation could be a molecular biomarker of cancer risk in smokers and that it could also serve as an intermediate endpoint in early-phase chemoprevention trials. Validation of these initial results is clearly needed, both for the utility of PI3K activation as a risk marker and as an efficacy marker. However, the approach of using high-throughput analyses such as gene expression arrays to gauge the effect of interventions on at-risk epithelia (or their surrogates) presents new opportunities for performing smaller, more efficient, and more informative cancer prevention clinical trials.

A number of other promising agents are being evaluated for lung cancer prevention. These include a prostacyclin analog, iloprost, which has been shown to decrease the severity of endobronchial dysplasia in former smokers in a phase IIb clinical trial and continues to be evaluated further (51). Of note, iloprost was not effective in current smokers, underscoring the concept that different interventions may be needed for current versus former smokers. Preclinical data indicate that the mechanism of action of iloprost involves the activation of PPAR-γ, which is the target of the thiazolidinedione class of antidiabetic agents (52). Pioglitazone, a member of this class of drugs, is approved for the treatment of diabetes and is also in phase II clinical trials for both lung cancer and oral cancer prevention. Other agents being studied in early-phase clinical trials include green tea catechins, erlotinib, isothiocyanates, and metformin.

## Conclusions—The Path Forward

Tremendous progress has been made in our understanding of the molecular effects of tobacco-induced carcinogenesis since the Surgeon General's report linking cigarette smoking with lung cancer 50 years ago. Translation of this knowledge to the identification of the truly high-risk individuals and ways of preventing or detecting lung cancer early is a "work in progress," but one that is already beginning to have real-world applications. The demonstration that early detection can significantly reduce the risk of lung cancer death now provides the opportunity to identify higher risk populations for whom screening would be most beneficial. Incorporation of biomarkers of tobacco exposure and detoxification capabilities, as well as CT characteristics, into risk prediction models may offer a more personalized approach for risk assessment. Promising chemopreventive approaches can then be tested in the cohorts for whom the risk–benefit balance is most favorable. Although plenty of challenges still exist, the palpable progress offers hope that disseminated lung cancer will become a preventable disease.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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## Authors' Contributions

**Conception and design:** S.S. Hecht, E. Szabo
**Writing, review, and/or revision of the manuscript:** S.S. Hecht, E. Szabo
**Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases):** S.S. Hecht, E. Szabo

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