



## Chemoprevention of Lung Cancer

### Diagnosis and Management of Lung Cancer, 3rd ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines

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**Background:** Lung cancer is the most common cause of cancer death in men and women in the United States. Cigarette smoking is the main risk factor. Former smokers are at a substantially increased risk of developing lung cancer compared with lifetime never smokers. Chemoprevention refers to the use of specific agents to reverse, suppress, or prevent the process of carcinogenesis. This article reviews the major agents that have been studied for chemoprevention.

**Methods:** Articles of primary, secondary, and tertiary prevention trials were reviewed and summarized to obtain recommendations.

**Results:** None of the phase 3 trials with the agents  $\beta$ -carotene, retinol, 13-*cis*-retinoic acid,  $\alpha$ -tocopherol, N-acetylcysteine, acetylsalicylic acid, or selenium has demonstrated beneficial and reproducible results. To facilitate the evaluation of promising agents and to lessen the need for a large sample size, extensive time commitment, and expense, surrogate end point biomarker trials are being conducted to assist in identifying the most promising agents for later-stage chemoprevention trials. With the understanding of important cellular signaling pathways and the expansion of potentially important targets, agents (many of which target inflammation and the arachidonic acid pathway) are being developed and tested which may prevent or reverse lung carcinogenesis.

**Conclusions:** By integrating biologic knowledge, additional early-phase trials can be performed in a reasonable time frame. The future of lung cancer chemoprevention should entail the evaluation of single agents or combinations that target various pathways while working toward identification and validation of intermediate end points. *CHEST 2013; 143(5)(Suppl):e40S–e60S*

**Abbreviations:** AAH = atypical adenomatous hyperplasia; ACCP = American College of Chest Physicians; ADT = anethole dithiolethione; ATBC =  $\alpha$ -Tocopherol  $\beta$ -Carotene; CARET =  $\beta$ -Carotene and Retinol Efficacy Trial; COX = cyclooxygenase; EGFR = epidermal growth factor receptor; HOPE = Heart Outcomes Prevention Evaluation; LOX = lipoxygenase; mTOR = mammalian target of rapamycin; NSCLC = non-small cell lung cancer; PGE<sub>2</sub> = prostaglandin E<sub>2</sub>; PGI<sub>2</sub> = prostacyclin; PI3K = phosphoinositide 3-kinase; PPAR $\gamma$  = peroxisome proliferator-activated receptor  $\gamma$ ; RR = relative risk

#### SUMMARY OF RECOMMENDATIONS

**3.1.1.1. For individuals with a greater than 20 pack year history of smoking or with a history of lung cancer, the use of  $\beta$  carotene supplementation is not recommended for primary, secondary, or tertiary chemoprevention of lung cancer (Grade 1A).**

*Remarks:* The dose of  $\beta$  carotene used in these studies was 20-30 mg per day or 50 mg every other day.

**3.5.1.1. For individuals at risk for lung cancer and for patients with a history of lung cancer, the use of vitamin E, retinoids, and N-acetylcysteine and isotretinoin is not recommended for primary, secondary, or tertiary prevention of lung cancer (Grade 1A).**

**3.6.1.1. For individuals at risk for lung cancer and for patients with a history of lung cancer, the use of aspirin is not recommended for primary, secondary, or tertiary prevention of lung**

cancer (outside of the setting of a well-designed clinical trial) (Grade 1B).

**3.7.1.1. In individuals with a history of early stage non-small cell lung cancer (NSCLC), the use of selenium as a tertiary chemopreventive agent of lung cancer is not recommended (Grade 1B).**

**5.7.1. For individuals at risk for lung cancer or with a history of lung cancer, prostacyclin analogs (iloprost), cyclooxygenase-2 inhibitors (celecoxib), and anethole dithiolethione, are not recommended for use for primary, secondary, or tertiary lung cancer chemoprevention (outside of the setting of a well-designed clinical trial) (Grade 1B).**

**5.7.2. For individuals at risk for lung cancer or with a history of lung cancer, inhaled steroids are not recommended for use for primary, secondary, or tertiary lung cancer chemoprevention (outside of the setting of a well-designed clinical trial) (Grade 1B).**

**6.7.1. For individuals at risk for lung cancer or with a history of lung cancer, the use of**

**pioglitazone or myoinositol, for primary, secondary, or tertiary lung cancer chemoprevention is not recommended (outside of the setting of a well-designed clinical trial) (Grade 1B).**

**6.7.2. In individuals at risk for lung cancer, the use of tea extract, or metformin is not suggested for primary, secondary or tertiary prevention of lung cancer (outside of the setting of a well-designed clinical trial) (Grade 2C).**

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The number of newly diagnosed cases of lung cancer in the United States in 2012 was estimated to be 226,160. Lung cancer causes more deaths (160,340) than colorectal cancer, breast cancer, and prostate cancer combined.<sup>1</sup> Estimated 2011 new lung cancer cases worldwide were 1,608,800, and there will be an estimated 1,387,400 deaths.<sup>2</sup> The single most important modifiable risk factor for lung cancer is smoking. Today 19.3% of the adult population in the United States continues to smoke, and since 2002 there have been more former smokers than current smokers in the United States.<sup>3</sup> In those who actively smoke, the risk of lung cancer is, on average, 10-fold higher than in lifetime never smokers (defined as a person who has smoked < 100 cigarettes). Although smoking prevention and cessation remain essential in the overall strategy for lung cancer prevention, former smokers continue to have an elevated risk of lung cancer for years after quitting.<sup>4</sup> In fact, more than one-half of lung cancers occur in individuals who have stopped smoking.<sup>5</sup>

The 5-year US survival rate for lung cancer is a discouraging 16%,<sup>1</sup> and although there has been an interval improvement in survival, the survival advances realized in other common malignancies have not translated to lung cancer. One reason for the discouraging survival statistics is that most patients present with late-stage disease. With advances in biologically targeted therapeutic agents, the treatment of advanced lung cancer should continue to make incremental improvements,<sup>6</sup> but effective chemopreventive agents are sorely needed. Efforts at improving this dismal outcome have been directed more recently at chemoprevention to reduce the incidence and mortality of lung cancer. Clinical experience has shown that chemopreventive agents may have dramatically different results in current and former smokers<sup>7</sup> and that many trials either exclude current smokers or analyze these subjects separately.

### *Carcinogenesis*

The rationale for chemoprevention is based on two main concepts, multistep carcinogenesis and “field cancerization.” These concepts can be used to explain the

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process of lung tumorigenesis as it occurs over time and throughout the entire bronchoalveolar epithelium.<sup>8,9</sup>

Multistep carcinogenesis derives from the theory that the progression of normal bronchoepithelial cells to a malignant lesion entails multiple steps involving numerous morphologic and molecular modifications. Similar to many solid organ tumors, lung tumorigenesis results from a series of genetic and epigenetic alterations in pulmonary epithelial cells. The World Health Organization classification for lung cancer recognizes distinct histologic lesions that can be reproducibly graded as precursors of non-small cell lung cancer (NSCLC).<sup>10</sup> A series of alterations occur over time that lead to malignant transformation with unregulated clonal expansion and cellular proliferation. The morphologic correlate of multistep carcinogenesis is the progression of bronchial epithelium from reserve cell hyperplasia to squamous metaplasia, and then to increasing grades of dysplasia (mild, moderate, and severe), culminating in carcinoma in situ and invasive squamous cell carcinoma. Lung adenocarcinoma is also preceded by premalignant lesions called atypical adenomatous hyperplasia (AAH). Specific genetic abnormalities have been described that correlate with the morphologic steps involved in the evolution to malignancy.<sup>8</sup>

Physiologically, environmental factors (most notably tobacco smoke) injure the bronchial epithelium and this damage must be repaired. To control the proliferative response to tissue damage, a complex system of intercellular communication has evolved that includes epithelial cells, stroma, and inflammatory cells.<sup>11</sup> The vehicles of communication are growth factors, cytokines, peptides, lipid metabolites, and their respective cellular receptors. Their functions include induction and suppression of proliferation, but also of migration, contact inhibition, angiogenesis, apoptosis, and antitumor immunity. Specific mutations in growth factor receptors (so-called “driver mutations”) are the hallmarks of certain lung cancer phenotypes and form the basis of molecularly targeted therapies.<sup>12</sup> This subject is covered in more detail by Nana-Sinkam and Powell<sup>13</sup> in the “Molecular Biology of Lung Cancer” article in the ACCP Lung Cancer Guidelines. Reactive oxygen species that are generated during inflammation can result in DNA damage and may thus trigger or accelerate carcinogenesis.

In 1953, it was established that many areas of the aerodigestive tract are simultaneously at risk of cancer formation due to carcinogen exposure.<sup>14</sup> According to this concept, known as “field cancerization,” histologically normal-appearing tissue adjacent to malignant lesions contains molecular abnormalities similar to those seen in tumor tissue.<sup>15</sup> This serves to explain the synchronous presence of various pre-

ligant and malignant lesions at different locations in the aerodigestive tract of the same at-risk individual. The high rate of second primary cancers (possibly as high as 2% to 3% per year)<sup>16</sup> in individuals who underwent curative treatment of an aerodigestive malignancy provides further evidence for field cancerization.

### *Tobacco Cessation and Prevention*

Tobacco exposure, including the use of smokeless tobacco, pipes, and cigars, is among the most preventable causes of morbidity and mortality in the United States. The most pertinent of these is cigarette smoke; the overwhelming majority of lung cancer is associated with cigarette smoking.<sup>17</sup> Given the harm associated with tobacco use, it is important not only to promote the cessation of tobacco use but also to prevent the initiation.

Preventing the use of tobacco, and, thus, reducing the incidence of smoking, plays an imperative role in public health (covered in more detail by Alberg et al<sup>38</sup> in the “Epidemiology of Lung Cancer” article in the ACCP Lung Cancer Guidelines). The key is to provide early information about the harms of tobacco exposure to middle school and high school students and to provide the tools to smokers that will allow them to quit. Policies and programs exist and continue to be developed to educate youth on the harms of tobacco use because of its potential for dependency and associated morbidity and mortality.

Advocacy efforts have been increasingly successful at limiting tobacco use and public exposure to environmental tobacco smoke. Some of these methods include strict regulation of tobacco advertisements, increases in tobacco taxes, and comprehensive smoking bans for indoor and outdoor public areas.

Tobacco cessation remains another major public health focus in the United States. Numerous cessation programs are available for those interested in quitting, and the Centers for Disease Control and Prevention estimate that in 2010, 68.8% of adult smokers wanted to stop smoking and 52.4% had made an attempt to quit during the previous year,<sup>3</sup> using methods ranging from behavioral therapy to pharmacologic interventions (covered in more detail by Alberg et al<sup>38</sup> in the “Epidemiology of Lung Cancer” article in the ACCP Lung Cancer Guidelines). An essential aspect of all primary care practices should be to ask all patients about smoking status, with counseling and advice provided. These strategies have been associated with an increase in smoking cessation.<sup>18</sup> By providing mutual support and advice on behavior modifications and coping skills, group therapy has also been found to be an effective method.<sup>19</sup> The use of pharmacologic interventions, such as all forms of nicotine replacement (including nicotine spray, gum,

and patches) and bupropion, and varenicline (partial agonists of nicotinic acetylcholine receptors), has been effective in increasing smoking cessation rates.<sup>20-22</sup> Other techniques, such as acupuncture and hypnosis, have not shown efficacy to date.<sup>23</sup>

Smoking cessation can result in a decrease in precancerous lesions from 27% to 7%.<sup>24</sup> For those who have not smoked for 10 years, the risk of lung cancer may be 30% to 50% lower than that of current smokers.<sup>4,24</sup> Unfortunately, unlike other tobacco-related diseases in which the increased risk gradually declines to that approaching a never smoker, lung cancer risk will remain permanently elevated in former smokers. The number of former smokers who develop lung cancer suggests that a subgroup of former smokers sustains extensive lung damage that is not repaired after smoking cessation.

The only intervention that has been shown to be effective in reducing the risk of lung cancer is smoking cessation, demonstrated prospectively in the Lung Health Study. In this large study, smokers were assigned to smoking cessation intervention programs or were not assigned to smoking cessation intervention, with a resultant 55% reduction in lung cancer risk observed in successful quitters.<sup>25</sup>

Smoking cessation is clearly the most effective intervention to reduce lung cancer risk, and many options are available to help. Although physicians are strongly encouraged to discuss these options with their patients to develop individualized cessation plans, it should be noted that most lung cancers still occur in those who have stopped smoking. To help reduce the incidence of lung cancer, continued efforts have evaluated chemopreventive interventions to modify cancer risk, a topic covered in more detail by Socinski et al<sup>175</sup> in the "Treatment of Tobacco Use in Lung Cancer" article in the ACCP Lung Cancer Guidelines.

### *Chemoprevention*

Chemoprevention is defined as the use of dietary or pharmaceutical interventions to slow or reverse the progression of premalignancy to invasive cancer.<sup>26</sup> Chemoprevention as a means of reducing cancer incidence has been successful for skin (basal cell carcinoma in basal cell nevus syndrome),<sup>27</sup> breast,<sup>28</sup> and prostate cancer.<sup>29</sup> Because lung carcinogenesis can evolve over 20 to 30 years, the feasibility of large lung tumor prevention trials is limited. More recent studies have appropriately chosen to evaluate the effects of intervention on intermediate end points (endobronchial histology) and inhibition of the carcinogenic progression. The methodology employed to review articles and grade recommendations is summarized by Lewis et al<sup>30</sup> in the "Methodology for Development of Guidelines for Lung Cancer" article in the

ACCP Lung Cancer Guidelines. It is organized into the following sections: (1) high-risk populations and how agents are selected for study, (2) agents tested in previous trials with lung cancer as the end point, (3) intermediate end point biomarkers and novel methods for chemoprevention trials, (4) agents tested in intermediate end point biomarker trials, and (5) conclusions/future directions.

## 1.0 METHODS

In 2011-2012, a panel of experts corresponded to update the previous recommendations for the chemoprevention of lung cancer. The panel consisted of investigators experienced in the formulation, design, and execution of chemoprevention clinical trials. Disagreements were resolved to establish guidelines for practitioners to use for patients at high risk of developing lung cancer.

To come to a consensus on the various lung cancer chemoprevention guidelines presented in this topic, a systematic review of the literature was performed (detail provided by Lewis et al<sup>30</sup> in the "Methodology for Development of Guidelines for Lung Cancer" article in the ACCP Lung Cancer Guidelines search strategy and results available on request). The searches were structured around the population, intervention, comparison, outcome (PICO) question (see also Table S1): Are there agents beyond smoking cessation that are effective in reducing second primary tumor rates among current smokers with a history of early-stage lung cancer treated with definitive surgery? Topic panelists conducted their literature searches in PubMed. Searches were not limited by publication date and, at the minimum, covered the years 2005 to 2011 (to cover the timeframe following the prior American College of Chest Physicians Lung Cancer Guidelines). These guidelines were focused on primary, secondary, and tertiary lung cancer chemoprevention studies, most of which were funded by the National Cancer Institute. To establish study quality, recommendations were organized by the panel of experts on the writing committee and then graded by the standardized American College of Chest Physicians methods (see the "Methodology for Development of Guidelines for Lung Cancer" article in the ACCP Lung Cancer Guidelines<sup>30</sup>). Prior to final approval, this topic was reviewed by all the panel members,<sup>30</sup> which included a multidisciplinary team consisting of thoracic surgeons, medical oncologists, radiation oncologists, and pulmonologists, followed by several additional levels of review as described by Lewis et al<sup>30</sup> the "Methodology for Development of Guidelines for Lung Cancer" article in the ACCP Lung Cancer Guidelines.

## 2.0 KEY CONSIDERATIONS IN DESIGNING CHEMOPREVENTION TRIALS FOR LUNG CANCER

### *2.1 Identification of Candidate Agents*

The selection of appropriate targets for chemopreventive interventions requires a careful risk-benefit analysis that balances efficacy with potential harms related to the intervention. Indications of effectiveness are derived from knowledge of mechanisms, preclinical (in vitro and in vivo) experimental data, epidemiologic studies, and existing data from clinical trials, either early-phase cancer prevention trials or secondary analyses from trials performed for other indications.<sup>31</sup> Ongoing advances in the understanding

of lung cancer biology have resulted in the development of agents that target specific cellular pathways thought to be critical for tumor development and progression. This allows for therapies to be directed toward specific molecular abnormalities on which some cancers appear to be critically dependent for survival, a concept known as “oncogene addiction.”<sup>32</sup> However, the targetable molecular abnormalities, such as the epidermal growth factor receptor (EGFR) and the echinoderm microtubule-associated protein-like 4-anaplastic lymphoma kinase, have thus far been found primarily in lung cancers that occur much more commonly in never or light smokers. As appealing as the concept of molecularly targeted chemoprevention is, a deeper understanding of the molecular basis of tobacco-induced lung carcinogenesis is needed before targeted therapies can make an impact on the prevention of smoking-related lung cancer.

The other major consideration for agent selection for chemoprevention trials is the safety profile.<sup>33</sup> Because a chemopreventive intervention may need to be used for prolonged periods of time in a putatively healthy, albeit at-risk, population, it needs to be both safe and tolerable. In the setting of prevention, neither the major side effects that threaten an individual’s short-term or long-term well-being (such as the increased risk of cardiovascular disease associated with the use of the cyclooxygenase (COX)-2 inhibitor rofecoxib<sup>34</sup>) nor the minor side effects that interfere with compliance or significantly decrease quality of life are acceptable. Efforts to improve the toxicity profile of preventive interventions include the use of additional agents to counteract side effects (for instance, the use of proton pump inhibitors to counteract the GI toxicity of nonsteroidal antiinflammatory drugs), the use of combinations of agents that allow lower doses of the individual agents to be used (with potential benefits in terms of tolerability as well as improved efficacy), and alternative dosing schedules that minimize toxicity. Intermittent dosing has been modeled in animal systems with excellent preservation of efficacy, but human clinical trials have yet to be performed.<sup>35</sup> The need to optimize the risk-benefit balance requires the identification of high-risk individuals who stand to gain the most from chemopreventive interventions.

## *2.2 Recruitment and Retention of Subjects in Chemoprevention Trials*

Introducing chemoprevention is made difficult by the fact that smoking and the subsequent smoking-related diseases are increasingly seen in those of lower socioeconomic status. Statistics show that a greater proportion of college- and university-educated individuals have quit smoking, leaving a predominance

of lesser-educated individuals as today’s smokers.<sup>18</sup> Thirty percent of people without a high school degree and only 6% of people with a graduate degree are smokers. Thirty-one percent of people below the federal poverty line smoke, compared with 20% of people at or above the poverty line. Smokers are more likely to have poor health habits, including attitudes and beliefs about health care that differ from those of the general public. For example, in a study assessing beliefs about lung cancer screening, smokers reported that they undergo recommended screening for other types of cancer less often than their non-smoking counterparts, have less comprehension of what effective screening means, and were less likely to correctly identify a primary care physician.<sup>36</sup> They are also less likely to take part in the diagnostic tests required after a screen-detected abnormality for a variety of reasons, which include the personal cost in time, travel, out-of-pocket expenses, lost income, and failure to understand the importance of the diagnostic procedures.<sup>37</sup>

Chemoprevention trial messages are communicated more effectively to well-educated individuals through the use of print and electronic media than to those with lesser education who do not have an adequate understanding of what a chemoprevention program intends to accomplish. In addition, it may be difficult to reach those whose primary language is not English, those who are mistrustful of the public health-care system, those who come from very different health-care systems, and those who are socially disadvantaged. This is particularly true of new immigrant populations who tend to congregate with fellow immigrants and, through satellite connections, retain their connection to their homeland and its local media. The wide variety of languages used poses challenges to communicating the message of the benefit of chemoprevention. Educational materials for chemoprevention need to be created in multiple languages, using many different media that speak to the various cultures. If an individual is to take part in a study, his or her primary care physician must communicate the importance of participating in chemoprevention trials and complying with study protocols. It is important for primary care physicians and other health-care professionals to possess an accurate summary of current evidence to appropriately advise their patients, particularly former smokers who may not understand why they may still be at increased risk of lung cancer despite smoking cessation.

Recruitment and retention also depend on the complexity of the clinical trial protocol. Studies that involve a bronchoscopy and biopsy are generally more complicated than studies that involve a blood or sputum test or a low-dose chest CT scan. Future phase 2b/3 chemoprevention trials may benefit from

integration with an early lung cancer detection program involving low-dose thoracic CT scanning.

### 2.3 Principles of Chemoprevention

Chemoprevention involves the use of dietary or pharmaceutical interventions to slow or reverse the progression of premalignancy to invasive cancer.<sup>26</sup> Chemoprevention studies are subdivided into three distinct areas (primary, secondary, and tertiary). Each requires the recruitment of different risk groups. In primary prevention trials, subjects show no evidence of lung cancer but are at high risk (eg, current or former smokers who have COPD); for instance, those with a significant smoking history, family history, and COPD (lung cancer risk factors are covered in more detail by Alberg et al<sup>38</sup> in the “Epidemiology of Lung Cancer” article in the American College of Chest Physicians Lung Cancer Guidelines). Secondary prevention studies involve participants who have risk factors and evidence of premalignancy, such as sputum atypia or dysplasia on endobronchial biopsy specimens or AAH on transthoracic needle biopsy. Tertiary chemoprevention studies focus on the development of a second primary tumor (in this case, lung cancer) in subjects with a previous tobacco-related aerodigestive cancer.

Because 85% to 90% of lung cancers are associated with tobacco smoking, preventing the onset of smoking and bringing about successful smoking cessation in current smokers will most effectively achieve primary prevention of the disease. However, former heavy smokers carry a residual risk of lung cancer even years after smoking cessation; they make up >50% of the lung cancers diagnosed now,<sup>4,5,39</sup> and even long-term former smokers should be identified for chemoprevention trials.

Other factors also independently increase the risk of lung cancer. Examples of these are COPD,<sup>40,41</sup> environmental/occupational exposure to radon or asbestos,<sup>42</sup> or a family history of lung cancer. Genome-wide association studies have identified inherited susceptibility variants for lung cancer on several chromosomal loci, including 6q, 8q, 12q, and 15q.<sup>43-47</sup> (The genetic associations are discussed in more detail by Alberg et al<sup>38</sup> in the “Epidemiology of Lung Cancer” article in the ACCP Lung Cancer Guidelines). In general, the strength of the associations between non-smoking-related factors and lung cancer is substantially weaker than the association between cigarette smoking and lung cancer.

A history of lung cancer is associated with a 1% to 2% yearly risk of developing a new cancer.<sup>48</sup> The presence of sputum atypia is significantly associated with the development of cancer; it has been documented as preceding lung cancer development by 4 or

more years.<sup>49-52</sup> In one study, severe sputum atypia was associated with subsequent development of cancer in >40% of the population.<sup>53</sup> Varella-Garcia and colleagues<sup>54</sup> reported that the presence of chromosomal aneusomy in sputum cells was associated with an adjusted OR of lung cancer of 29.9. If confirmed in future studies, such a sputum-based approach to risk assessment offers tremendous potential to truly identify those who are at the highest risk and who might benefit the most from preventive interventions.

### 2.4 Identifying Highest-Risk Subjects for Trials

For clinical testing of promising agents, selecting high-risk cohorts reduces the sample size, the duration of the study, and the cost. In addition, this strategy optimizes the cost-benefit ratio for the study subjects. The selection of subjects with multiple lung cancer risk factors, such as  $\geq 20$  pack-years, moderate COPD, and evidence of endobronchial histologic changes, maximizes the use of resources for early-phase trials. It is important to recognize that individuals at high risk, such as active smokers, may have a different biology of disease from that of former smokers. As a result, the outcome of a trial may be adverse in one group (current smokers) yet beneficial in another (former smokers). In the end, the ultimate goal of lung cancer chemoprevention is to reduce disease incidence and mortality. Within each trial category, clear guidelines are needed for enrollment criteria.

Many studies have demonstrated that by stratifying by smoking exposure, the highest-risk subjects can be defined. For example, a longer smoking duration, a younger age of initiation, and a higher number of packs-per-day increase the risk of lung cancer.<sup>55</sup> There appears to be a continuum between risk and 10, 20, and 30 pack-years of smoking. No one level has been accepted as a definitive threshold of what is considered high risk. To identify smokers at highest risk, for interventions such as early detection and chemoprevention, several models have been developed using age, smoking history, and other risk factors. Examples of these are shown in Fig 1.<sup>42, 56-59</sup> With the exception of the model by Tammemagi,<sup>59</sup> which was based on a large population cohort of 38,254 ever smokers in the control arm of the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO), the discriminatory power and the accuracy of the prediction models are modest. In the Tammemagi model, the receiver operator characteristic area under the curves was 0.805 in the training set and 0.78 in external validation using 44,223 subjects in the intervention arm.<sup>59</sup> Further external validation of the Tammemagi model in a cohort of current and former smokers in British Columbia confirmed the accuracy of the prediction model.<sup>60</sup> The latter study also showed the incremental

FIGURE 1. [Section 2.4] Lung cancer risk prediction models.

Study/Year	Bach <sup>42</sup> /2003	Cassidy <sup>57</sup> /2008	Spitz <sup>58</sup> /2008	Tammemagi <sup>59</sup> /2011
Source	CARET Chemo-prevention Trial	Case control study	Case control study	PLCO trial
No.	14,000 smokers and 4,060 asbestos-exposed men	579 lung cancer and 1,157 non-lung cancer	1,851 lung cancer and 2,001 non-lung cancer	154,592 never smokers and ever smokers
Study population	Age 50-75, smoked 10-60 cigarettes/day for 25-55 years, quit < 20 years ago	Age 20-80, cancer >5 years ago can be included	Age 20-80	Healthy volunteers in general population, aged 55-74
<b>Significant risk factors</b>				
Age	+	+	+	+
Sex	+		+	
Asbestos exposure	+	+	+	
Smoking				
Cigarettes/day	+		+	+
Duration, years	+	+	+	+
Years stopped	+		+	+
Second hand smoke			+	
Family history		+		+
COPD			+	+
Prior malignancy		+		
Prior pneumonia		+		
No hay fever			+	
Education level				+
CXR <3 years ago				+
BMI				+
Discriminatory Power (AUC 95% CI)	0.66 (0.64-0.69)	0.69 (0.67-0.71)	0.69 (0.66-0.71)	0.809 (0.796-0.822)

AUC = area under the curve; BMI = body mass index; CARET =  $\beta$ -Carotene and Retinol Efficacy Trial; CXR = chest radiograph.

value of spirometry (FEV<sub>1</sub> % predicted) in lung cancer risk prediction in keeping with other studies that also found that lung function predicts lung cancer risk.<sup>61,62</sup> The addition of genes that are associated with either the healthy smokers (protective) or the lung cancer (susceptibility) phenotype may further improve the accuracy of risk prediction.<sup>63</sup> In a population that has already been screened with low-dose helical CT scans, incorporation of nodule characteristics, particularly nonsolid nodules, into the risk assessment prediction further identifies individuals at especially high risk.<sup>64</sup> The availability of accurate risk prediction models opens up the possibility of using lung cancer, instead of intermediate end point biomarkers, as the end point for chemoprevention trials.

### 3.0 AGENTS TESTED IN CHEMOPREVENTION TRIALS (WITH LUNG CANCER AS AN END POINT)

#### 3.1 $\beta$ -Carotene: Use in Former and Current Smokers and Those With Asbestos Exposure

Based on epidemiologic data, a diet rich in fruits and vegetables (at least three servings per day) is associated with a lower cancer incidence. The  $\alpha$ -Tocopherol  $\beta$ -Carotene (ATBC) study randomized 29,133 people to receive  $\alpha$ -tocopherol,  $\beta$ -carotene, both, or placebo.<sup>65</sup> Study participants were followed for 5 to 8 years.

The incidence of lung cancer in the study group was 18% higher than in the placebo group ( $P < .01$ ). A second trial evaluating  $\beta$ -carotene, the  $\beta$ -Carotene and Retinol Efficacy Trial (CARET), evaluated high-risk current and former smokers, with a >20-pack-year history of smoking ( $n = 14,254$ ) or asbestos exposure and a 15-pack-year smoking history ( $n = 4,060$ ). Study subjects were randomized to receive either a combination of  $\beta$ -carotene and vitamin A or placebo. The relative risk (RR) of lung cancer in the active treatment group was 1.28 (95% CI, 1.04-1.57;  $P = .02$ ). In a subgroup analysis, the RR of lung cancer in current smokers was 1.40 (95% CI, 1.07-1.87), whereas the RR in participants who were no longer smoking at the time of randomization was 0.80 (95% CI, 0.48-1.31).<sup>66</sup> Both these trials demonstrated a higher incidence of lung cancer in those who had received  $\beta$ -carotene, particularly among active smokers.

In the United States, the Physicians' Health Study evaluated 22,071 physicians, whose patients were randomized to  $\beta$ -carotene or placebo.<sup>67</sup> There was no difference in lung cancer rates in those who received the  $\beta$ -carotene. The Women's Health Study explored the use of 50 mg of  $\beta$ -carotene every other day vs placebo in 39,876 women aged 45 years or older. Thirteen percent of the women were smokers. The study was terminated early after a median treatment duration

of 2.1 years. There were no significant differences in the development of any site-specific cancer, including lung cancer (30 cases vs 21 cases).<sup>68</sup> Additionally, a meta-analysis of the large  $\beta$ -carotene trials (ATBC, CARET, Physicians' Health, and Women's Health) found an increased risk of lung cancer in current, not former, smokers who received  $\beta$ -carotene (OR, 1.21; 95% CI, 1.09-1.34).<sup>69</sup>

### 3.1.1 Recommendation

**3.1.1.1. For individuals with a greater than 20 pack year history of smoking or with a history of lung cancer, the use of  $\beta$  carotene supplementation is not recommended for primary, secondary, or tertiary chemoprevention of lung cancer (Grade 1A).**

*Remarks:* The dose of  $\beta$  carotene used in these studies was 20-30 mg per day or 50 mg every other day.

### 3.2 Vitamin E: Use in Men With a Smoking History

Epidemiologic data exist to support the premise that vitamin E ( $\alpha$ -tocopherol) has antitumor properties and that people with high levels of vitamin E are less likely to develop cancer. The ATBC study randomized > 29,000 high-risk participants to either  $\alpha$ -tocopherol,  $\beta$ -carotene, both, or placebo. The primary end point was a diagnosis of lung cancer, and the secondary end point was a diagnosis of any cancer. There was a nonsignificant reduction (2%) in the incidence of lung cancer.<sup>65</sup> The Heart Outcomes Prevention Evaluation (HOPE) and HOPE-The Ongoing Outcomes (HOPE-TOO) trials were international, randomized, double-blind, placebo-controlled trials, evaluating participants treated with daily 400 International Units of vitamin E vs placebo. The incidence of lung cancer did not differ between the vitamin E and the placebo arm, either in the primary analysis of the HOPE trial (69 [1.4%] vs 92 [2%],  $P = .04$ ) or in the HOPE-TOO trial (58 [1.6%] vs 74 [2.1%],  $P = .16$ ).<sup>70</sup> The Women's Health Study explored the use of 600 International Units of vitamin E every other day or placebo in 39,876 women 45 years or older with 10.1 years of follow-up. There was no significant difference in lung cancer incidence between the treatment and placebo arms (RR, 1.09; 95% CI, 0.83-1.44).<sup>71</sup>

### 3.3 Vitamin A: Use in Current or Former Smokers

Epidemiologic data supported the idea that the consumption of fruits and vegetables high in vitamin A could lower the incidence of lung cancer. The CARET randomized participants to vitamin A and  $\beta$ -carotene or placebo. Participants were either current smokers or smokers who had quit in the past

6 years. As summarized earlier, this study found an RR of 1.28 ( $P = .02$ ) for lung cancer in the treatment arm compared with the placebo arm.<sup>7</sup>

### 3.4 N-acetylcysteine

Preclinical studies have demonstrated that N-acetylcysteine, an antioxidant that prevents cellular damage, has antitumor properties. A large clinical study evaluated this agent in 1,023 patients with NSCLC (pT1-T3, N0-1, or T3, N0) treated with curative intent.<sup>72</sup> Subjects were randomized to N-acetylcysteine, vitamin A (retinyl palmitate), both, or neither. No significant differences were noted among the groups for the primary end points of tumor recurrence, death, or second lung cancer.

### 3.5 Isotretinoin (13-cis Retinoic Acid)

#### Use in Patients With Stage I NSCLC

Preclinical and early clinical studies have suggested that retinoids have chemopreventive effects. A large randomized trial of 1,166 subjects with stage I NSCLC treated with curative intent were randomized to receive placebo or isotretinoin.<sup>73</sup> The hazard ratio was 1.08 (95% CI, 0.78-1.49) for time to second primary tumor, 0.99 (95% CI, 0.76-1.29) for recurrence, and 1.07 (95% CI, 0.84-1.35) for mortality. Isotretinoin did not decrease the incidence of second primary tumors, recurrence rate, or mortality from lung cancer.

#### 3.5.1 Recommendation

**3.5.1.1. For individuals at risk for lung cancer and for patients with a history of lung cancer, the use of vitamin E, retinoids, and N-acetylcysteine and isotretinoin is not recommended for primary, secondary, or tertiary prevention of lung cancer (Grade 1A).**

### 3.6 Acetylsalicylic Acid

Ample research supports a protective role of acetylsalicylic acid (ASA) (aspirin) and nonsteroidal antiinflammatory drugs in preventing cancer development. Three major trials have been conducted to evaluate the use of aspirin in lung cancer prevention. The UK Physicians' Health Study was a 6-year, randomized trial that evaluated 5,139 healthy male doctors who were receiving 500 mg/d aspirin. Eleven percent were current smokers, and 39% were former smokers. The lung cancer death rate in the aspirin group was 7.4 per 10,000 person-years compared with 11.6 per 10,000 person-years in the placebo group, a difference that was not statistically significant.<sup>74</sup> In the United States, the Physicians' Health Study involved 22,071 physicians to determine whether low-dose aspirin (325 mg every other day) decreases cardiovascular

mortality and whether  $\beta$ -carotene reduces the incidence of cancer. No reduction in lung cancer rate was found in participants who had taken aspirin.<sup>75</sup>

In the Women's Health Study, 39,876 women in the United States were randomized to receive either aspirin or placebo every other day. Approximately 13% were current smokers and 35.8% were former smokers. There was an average of 10.1 years of follow-up. Lung cancer was a secondary end point and developed in 205 participants. The RR of lung cancer in the aspirin group was 0.78, which did not reach statistical significance.<sup>76</sup> In addition, aspirin failed to prevent colorectal adenomas in this study, which is consistent with the findings of observational studies suggesting that daily aspirin is required for the prevention of cancer.<sup>77-79</sup>

Observational studies also suggest that the use of aspirin for at least 5 years is required before reductions in the risk of cancer are observed, and that the effect of aspirin on the risk of colorectal cancer on follow-up of randomized trials was greatest in patients with a duration of trial treatment of 5 years or longer.<sup>80</sup> These findings led to a meta-analysis of eight randomized controlled trials on the effect of daily aspirin vs control (conducted originally for the prevention of vascular events) on the risk of fatal cancer. The study found a 20% to 30% reduction in lung-cancer mortality in people taking daily aspirin for 5 or more years.<sup>81</sup> This benefit was limited to adenocarcinoma histology, and a similar benefit was noted in reducing mortality from cancers arising at multiple organ sites. Although highly encouraging, these data do not adequately address the balance of risks and benefits of aspirin in view of well-documented GI and bleeding side effects. Additional studies with long-term follow-up are needed to better address the role of aspirin in lung cancer prevention.

### 3.6.1 Recommendation

**3.6.1.1. For individuals at risk for lung cancer and for patients with a history of lung cancer, the use of aspirin is not recommended for primary, secondary, or tertiary prevention of lung cancer (outside of the setting of a well-designed clinical trial) (Grade 1B).**

### 3.7 Vitamin and Mineral Combinations

In 2006, the results from a study conducted in Linxian, China, were published. This study examined the effect of supplementation with four different combinations of vitamins and minerals in the prevention of lung cancer mortality in 29,584 healthy adults. The participants were randomly assigned to take either a vitamin/mineral combination or a placebo for 5 or more years. The combinations tested included

retinol and zinc, riboflavin and niacin, ascorbic acid and molybdenum, and  $\beta$ -carotene,  $\alpha$ -tocopherol, and selenium. Lung cancer deaths ( $n = 147$ ) identified during the trial period and 10 years after the trial ended were the primary study outcomes. This was the first study to simultaneously evaluate combinations of vitamins (other than  $\beta$ -carotene, retinol, and  $\alpha$ -tocopherol) and minerals for lung cancer chemoprevention. No significant differences in lung cancer death rates were found for any of the four combinations of supplements tested in this study.<sup>82</sup>

### 3.8 Selenium

The use of selenium for cancer prevention has been an area of considerable interest because it improves cellular defense against oxidative stress.<sup>83</sup> The Nutritional Prevention of Cancer trial tested selenium for the prevention of nonmelanoma skin cancer, and although it did not prevent skin cancer, the subjects in the trial exhibited a 44% decrease in lung cancer incidence.<sup>83</sup> Further analysis of the trial results determined that selenium supplementation decreased the lung cancer incidence only in the tertile with the lowest baseline plasma selenium levels.<sup>84</sup> Eastern Cooperative Oncology Group protocol E5597 was a phase 3, double-blind, placebo-controlled study of 200  $\mu\text{g}$  selenized yeast in the prevention of second primary lung cancers in patients who had a complete resection of early stage (T1/2N0M0) NSCLC.<sup>85</sup> This study was terminated early because of a nonsignificant trend between the treatment groups. In the larger Selenium and Vitamin E Cancer Prevention Trial (SELECT) for prostate cancer chemoprevention, lung cancer was a predetermined secondary end point. A total of 35,533 men were randomized to selenium (200  $\mu\text{g}$  L-selenomethionine) or vitamin E (400 International Units of  $\alpha$ -tocopherol). No significant differences regarding lung cancer incidence or mortality were seen between the treatment groups. One review investigated selenium as a lung cancer chemopreventive agent and concluded that it "should not be used as a general strategy for lung cancer prevention."<sup>86</sup>

### 3.8.1 Recommendation

**3.8.1.1. In individuals with a history of early stage NSCLC, the use of selenium as a tertiary chemopreventive agent of lung cancer is not recommended (Grade 1B).**

## 4.0 INTERMEDIATE END POINT BIOMARKERS IN CHEMOPREVENTION CLINICAL TRIALS

Randomized controlled phase 3 clinical trials represent the definitive way to demonstrate preventive

efficacy by assessing the ability of an intervention to affect cancer incidence and mortality. Phase 2 efficacy cancer prevention trials, on the other hand, rely on short-term (or intermediate) end points that are theoretically predictive of patient outcomes such as cancer incidence. In contrast to phase 3 cancer treatment trials that rely on tumor measurements to assess agent efficacy, phase 2 cancer prevention trials do not have easily measured primary end points for indicating preventive efficacy. Instead, early-phase cancer prevention trials generally assess surrogate efficacy measures such as histologic preneoplasia or proliferative indices, which are even more distantly related to the definitive end point of cancer incidence. The definitions for surrogate end point biomarkers and intermediate end point biomarkers are not identical. A surrogate end point is an end point that is obtained earlier, less invasively, or at a lower cost than a true end point and thus serves as a substitute for the true outcome. An intermediate end point augments, but does not necessarily replace, the true outcome.<sup>87-89</sup> In the context of this discussion, the term “intermediate end point biomarker” will be used because no surrogate end point biomarkers have been validated for use in lung cancer chemoprevention trials.

To be useful, an intermediate marker should satisfy several criteria.<sup>89-91</sup> The marker should be integrally involved in the process of carcinogenesis, and its expression should correlate with disease course. The expression of the marker should differ between normal and at-risk epithelia and it should be easily and reproducibly measurable in specimens obtained in clinical trials. Last, the expression of the marker should be modulated by effective interventions, and there should be minimal spontaneous fluctuations and no modulation by ineffective agents. A marker that satisfies these criteria must be validated in prospective clinical trials.<sup>88-89</sup>

Intermediate end points can significantly inform early-phase drug development by demonstrating that the interventions affect the target epithelium. The most commonly used intermediate end point in lung cancer phase 2 trials is bronchial dysplasia, the precursor to squamous cell carcinoma obtained by bronchoscopic biopsy. The natural history of such lesions can vary significantly from lesion to lesion. Approximately 3.5% of low or moderate dysplasias progress to severe dysplasia, 37% of severe dysplasias remain or progress (vs regress to a lower grade lesion), and approximately 50% of carcinomas in situ progress to invasive carcinoma within a 2- to 3-year follow-up period.<sup>92,93</sup> Because one cannot predict which dysplastic lesions will persist or progress, lung cancer prevention clinical trials, even at the phase 2 level, should ideally be randomized and placebo controlled. This design strategy allows the “spontaneous” reversion rate in the placebo arm to be used for comparison

with account for issues related to the effects of multiple biopsies and for true biologic reversion. A number of phase 2 trials have used this approach, which is discussed in the section that follows. Further analysis of these preinvasive lesions, such as the proteomic analysis described by Hassanein and colleagues,<sup>94</sup> may provide more accurate information as to which individuals with dysplasia will progress to cancer.

Whereas bronchial dysplasia is a recognized premalignant condition associated with squamous cell lung carcinoma, the premalignancy associated with adenocarcinomas is believed to be AAH.<sup>95</sup> AAH is generally diagnosed incidentally during resection for lung cancer, but with the increasing use of helical CT scan screening, ground-glass opacities, some of which represent AAH, are being identified more often. The incidence of AAH in resections of ground-glass opacities varies widely in the literature, from 6% to 58%.<sup>96,97</sup> However, because resected nodules represent lesions that are suspicious enough to merit surgery, it is possible that persistent, small, subcentimeter nodules that are identified incidentally or by screening CT scan may be enriched for AAH. Whether CT scan-detected lung nodules can serve as intermediate end points for chemoprevention trials is now being investigated. Veronesi and colleagues<sup>98</sup> performed the first such trial using inhaled budesonide in smokers with persistent indeterminate lung nodules, as discussed later. Although the overall results were negative, the majority of the nodules were solid and did not change over 1 year of intervention. In contrast to the placebo group, a nonsignificant decrease in the size of the ground-glass opacities was noted with the intervention group, although the inability to histologically confirm the cause of the lesions was an important limitation of the study. Whether CT scan-detected ground-glass opacities can serve as intermediate end points for adenocarcinoma prevention trials requires additional study.

A variety of other biomarkers, such as the Ki-67 labeling index (a marker of cellular proliferation), have also been used as primary study end points, although the direct correlation between such biomarkers and cancer incidence is even more remote than the relationship between intraepithelial neoplasia and cancer.<sup>90</sup> Given the absence of tumor end points, prevention trials generally assess proliferation in the preneoplastic or histologically normal epithelium in high-risk individuals, a setting in which proliferation is elevated, but to a far lesser degree than in overt malignancy. In separate phase 2b trials, Kim et al<sup>99</sup> and Mao et al<sup>100</sup> demonstrated a statistically significant decrease in bronchial Ki-67 in heavy and former smokers treated with celecoxib. Although proliferation appears to be more sensitive to modulation than histology, in the absence of data linking changes in

proliferation with the gold standard end point of cancer incidence, it is difficult to know how predictive this end point will prove to be.

An alternative to using single markers (such as Ki-67) is to examine a panel of markers or even a gene expression profile. Gustafson and colleagues<sup>101</sup> assessed gene expression signatures in the normal bronchial epithelium and reported that the phosphoinositide 3-kinase (PI3K) pathway is upregulated early during lung carcinogenesis and that intervention with the drug myo-inositol inhibited activated PI3K signaling in correlation with the regression of dysplasia. The use of gene expression analysis to study a small number of subjects treated with an intervention offers a faster and more efficient way to evaluate the mechanisms of action of the intervention and to provide evidence of efficacy. Such an approach has the potential to replace the relatively large phase 2b trials that study 100 or more participants with interventions lasting 3 to 6 months or more. Other high throughput analyses, such as ones assessing DNA methylation or microRNA profiles, need to be examined in future studies. Again, in the absence of data linking these changes with changes in cancer incidence, it is difficult to know the impact of these intermediate end points on future studies.

## 5.0 AGENTS TESTED IN INTERMEDIATE END POINT BIOMARKER TRIALS

### 5.1 Arachidonic Pathway and Lung Cancer Chemoprevention

Arachidonic acid is metabolized to prostaglandins and prostacyclin (PGI<sub>2</sub>) by the COX pathway, whereas leukotrienes are formed via the lipoxygenase (LOX) pathway. Their end products have been closely linked to carcinogenesis.<sup>102</sup> Two isoforms of COX exist: COX-1 and COX-2. COX-1 exists in most cells and is constitutively active. In contrast, COX-2 is induced by inflammatory and mitogenic stimuli that lead to increased prostaglandin formation in inflamed and neoplastic tissues.<sup>102</sup> Despite having similar structures, COX-2 can be selectively inhibited.

Evidence exists to support arachidonic pathway modulation for the inhibition of carcinogenesis. Corticosteroids are known modulators of the eicosanoid-signaling pathway. Synthesized glucocorticoids have been demonstrated to block the development of cancer in A/J mice with induced pulmonary adenomas.<sup>103</sup> COX-2 expression has been demonstrated in premalignant and malignant bronchial cells<sup>104</sup>; higher levels are associated with a poor prognosis in those with NSCLC.<sup>104</sup> Preclinical murine models using the COX-2 inhibitor celecoxib have yielded conflicting results, with one study showing that celecoxib did not reduce the number or the size of the metastases,<sup>106</sup> and another

study reporting a decrease in the rate of growth of lung cancer and the number and the size of the metastases.<sup>107</sup> The expression of COX-2 has been shown to enhance tumorigenesis by regulation of angiogenesis,<sup>108</sup> invasion,<sup>109</sup> apoptosis,<sup>110</sup> and antitumor immunity.<sup>111</sup> However, not all preclinical carcinogenesis models have shown chemopreventive efficacy.<sup>106</sup>

5-LOX is an enzyme involved in the conversion of arachidonic acid to leukotrienes. Leukotrienes are proinflammatory and enhance cell adhesion.<sup>112</sup> They appear to impact the development and progression of lung cancer, based on data demonstrating that 5-LOX is expressed in lung cancers,<sup>113</sup> that 5-LOX inhibitors reduced the multiplicity and incidence of lung tumors in mice,<sup>114</sup> and that 5-LOX metabolites may play a role in angiogenesis.<sup>115</sup>

*5.1.1 Lung Cancer Chemoprevention Trials With Arachidonic Acid Pathway Modulators:* Several studies have been completed and others are ongoing to evaluate the use of arachidonic acid pathway modulators for lung cancer chemoprevention. The following is an overview of these trials.

### 5.2 Celecoxib

Ample preclinical data suggest that the COX-2/prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) signaling pathway plays a pivotal role in conferring the malignant phenotype.<sup>116</sup> Overproduction of PGE<sub>2</sub> (predominantly generated by the upregulation of COX-2) is associated with a variety of well-established carcinogenic mechanisms.<sup>117-119</sup> In animal models, the inhibition of COX-2 and PGE<sub>2</sub> synthesis suppresses lung tumorigenesis.<sup>120</sup> These data support the antineoplastic effect of COX-2 inhibitors and provide the rationale for evaluating their potential as chemoprevention agents for bronchogenic carcinoma.

The first randomized controlled trial with celecoxib was published in 2010.<sup>99</sup> Current or former smokers with at least a 20 pack-year smoking history were randomized into one of four treatment arms (3-month intervals of celecoxib then placebo, celecoxib then celecoxib, placebo then celecoxib, or placebo then placebo) and underwent serial bronchoscopy. The primary end point was modulation, by celecoxib, of bronchial Ki-67 expression (a cellular proliferation marker) from baseline to 3 months. High-dose celecoxib (400 mg bid) significantly decreased Ki-67 labeling in former smokers and current smokers compared with placebo after adjusting for metaplasia and smoking status ( $P = .02$ ), with stronger reduction of Ki-67 observed in former smokers.

Results from a second randomized controlled trial with celecoxib were published more recently.<sup>100</sup> In a phase 2a single-arm trial of celecoxib for lung cancer prevention in active smokers, celecoxib downregulated

PGE<sub>2</sub> and IL-10 production in alveolar macrophages,<sup>121</sup> and 6 months of celecoxib treatment significantly downregulated Ki-67 in endobronchial biopsies from heavy smokers.<sup>122</sup> These results were followed-up with a phase 2b randomized controlled trial of celecoxib for lung cancer prevention in former smokers.<sup>100</sup> Former smokers were recruited and randomized into two arms (6 months of 400 mg bid celecoxib then placebo, 6 months of placebo then celecoxib). The primary end point was the bronchial Ki-67 labeling index. Celecoxib significantly decreased the Ki-67 labeling index by an average of 34%, whereas placebo increased the Ki-67 labeling index by an average of 3.8% ( $P = .04$ ). Celecoxib did not have a significant effect on histopathology outcomes.

This study also incorporated low-dose helical CT scanning for baseline screening and secondary end point assessment at 12 months in 76 participants who had crossed over to the other study arm at 6 months (all of whom had received 6 months of celecoxib at the end of a 12-month treatment period). The decreases in the Ki-67 labeling index correlated with a reduction and/or resolution of lung nodules on chest CT scan. This study suggests that a systemically administered agent may be capable of globally impeding the driving force of carcinogenesis in the lungs, beyond the central airways.

### 5.3 Iloprost

PGI<sub>2</sub> is a prostaglandin H<sub>2</sub> metabolite with anti-inflammatory, antiproliferative, and potent antimetastatic properties.<sup>123,124</sup> Preclinical studies in transgenic mice with selective pulmonary PGI<sub>2</sub> synthase overexpression showed significantly reduced lung tumor multiplicity and incidence in response to either chemical carcinogens or exposure to tobacco smoke.<sup>125,126</sup> Iloprost, a long-lasting oral PGI<sub>2</sub> analog, also inhibited lung tumorigenesis in preclinical animal studies, and the effects were independent of the cell surface PGI<sub>2</sub> receptor.<sup>127</sup> These studies formed the basis of a National Cancer Institute-sponsored, double-blind, placebo-controlled clinical chemoprevention trial, in which subjects at high risk of lung cancer (sputum cytologic atypica, > 20 pack-years, known endobronchial dysplasia) received oral iloprost or placebo. The primary end point for the trial was endobronchial histology, and the trial showed that 6 months of oral iloprost significantly improved endobronchial dysplasia in former smokers.<sup>128</sup> Current smokers did not exhibit a treatment benefit. This was the first trial to show significant improvement in the primary end point of endobronchial histology. Additional studies using the iloprost biospecimens are now in progress to assist in identifying markers of response, and future trials are being planned.

### 5.4 Zileuton

A clinical trial of the 5-LOX inhibitor zileuton was conducted at the Karmanos Cancer Institute, in which the effect of zileuton on bronchial dysplasia (primary end point) and multiple molecular markers (secondary end points) in at-risk smokers or patients with curatively treated aerodigestive cancers was addressed. The results of this study are pending. A second study of zileuton, alone or in combination with celecoxib, recently finished accrual.<sup>129</sup>

### 5.5 Organosulfurs

The organosulfur compound anethole dithiolethione (ADT) is available in Europe and Canada for the treatment of xerostomia due to radiation. In 2002, a randomized, placebo-controlled, phase 2b trial evaluating ADT and placebo in smokers with bronchial dysplasia was conducted. The progression rate of pre-existing dysplastic lesions by two or more grades and/or the appearance of new lesions was significantly lower in the ADT group in both the person-specific (32% vs 59%) and lesion-specific (8% vs 17%) analyses.<sup>130</sup> A phase 3 trial of ADT was not conducted because of the abdominal bloating and flatulence associated with the study dose.

### 5.6 Budesonide

In mouse carcinogenesis model systems, the glucocorticoid budesonide, widely used for the treatment of asthma and COPD, inhibited lung carcinogenesis.<sup>131-133</sup> An epidemiologic study in 10,474 patients with COPD showed that treatment with high-dose inhaled steroids ( $\geq 1\ 200\ \mu\text{g}$  triamcinolone or its equivalent per day) decreased the risk of lung cancer after adjusting for age, smoking status, and smoking intensity (hazard ratio, 0.39; 95% CI, 0.16-0.96).<sup>134</sup> In a 6-month phase 2b clinical trial of inhaled budesonide (1,600  $\mu\text{g}$ ) vs placebo, budesonide had no effect on bronchial dysplasia or the prevention of new lesions, although it resulted in a modest decrease in p53 and *Bcl-2* protein expression in bronchial biopsy specimens and a slightly higher rate of resolution of CT scan-detected lung nodules.<sup>135</sup> Similarly, in a clinical trial of fluticasone (500  $\mu\text{g}$  bid) vs placebo for 6 months in subjects with endobronchial dysplasia, more subjects had a decrease and fewer had an increase in the number of CT scan-detected lung nodules in the fluticasone arm. However, this trend did not reach statistical significance.<sup>136</sup> A randomized, double-blind, placebo-controlled phase 2b trial of inhaled budesonide (800  $\mu\text{g}$  bid) was conducted in current and former smokers with CT scan-detected lung nodules present for at least 1 year. No significant difference in nodule size was observed between the budesonide and

placebo arms.<sup>98</sup> The per-lesion analysis revealed a significant effect of budesonide on the regression of existing target nodules ( $P = .02$ ) but a nonsignificant difference in the appearance of new lesions. The limitation of using CT scan-detected lung nodules as an intermediate end point is the lack of confirmation of the underlying histopathology. Only a small proportion of subcentimeter lung nodules are premalignant lesions (AAH).

## 5.7 Recommendations

**5.7.1. For individuals at risk for lung cancer or with a history of lung cancer, PGI<sub>2</sub> analogs (iloprost), COX-2 inhibitors (celecoxib), and anethole dithiolethione, are not recommended for use for primary, secondary, or tertiary lung cancer chemoprevention (outside of the setting of a well-designed clinical trial) (Grade 1B).**

**5.7.2. For individuals at risk for lung cancer or with a history of lung cancer, inhaled steroids are not recommended for use for primary, secondary, or tertiary lung cancer chemoprevention (outside of the setting of a well-designed clinical trial) (Grade 1B).**

## 6.0 ADDITIONAL TARGETED PATHWAYS/FUTURE DIRECTIONS

### 6.1 *Myo-inositol*

Myo-inositol is found in a wide variety of foods, such as whole grains, seeds, and fruits. It is a source of several second messengers, including diacylglycerol, and is an essential nutrient required by human cells for growth and survival in culture. Multiple animal studies show that myo-inositol inhibits carcinogenesis by 40% to 50% in both the induction and postinitiation phase.<sup>137,138</sup> In combination with budesonide, its efficacy is up to 80%.<sup>131</sup> Mechanistically, the PI3K pathway that is activated in the bronchial epithelial cells of smokers with dysplasia was found to be inhibited by myo-inositol and associated with regression of bronchial dysplasia.<sup>101</sup> The low toxicity and promising preclinical and phase 1 data led to further investigation of its chemopreventive effect in smokers at high risk of lung cancer (including second primary lung cancer) in an ongoing phase 2b randomized placebo-controlled trial in smokers with bronchial dysplasia.

### 6.2 *Peroxisome Proliferator-Activated Receptor $\gamma$ Agonists*

The peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ) is a member of the nuclear receptor superfamily consisting of steroid, retinoid, thyroid, vitamin D, and other receptors that dimerize and regulate gene transcription. PPAR $\gamma$  was initially identified as a key

regulator of adipogenic differentiation; the pleiotropic effects of PPAR $\gamma$  activation have been shown to include inhibition of proliferation, induction of apoptosis, induction of differentiation, inhibition of inflammation, and inhibition of metastasis in a variety of cancer cell lines and rodent carcinogenesis model systems (reviewed in Nemenoff et al<sup>139</sup> and Ondrey<sup>140</sup>). Ligands of PPAR $\gamma$  include eicosanoids and the thiazolidinedione class of antidiabetic agents, of which pioglitazone is currently approved and in use for the treatment of type 2 diabetes mellitus.

Multiple lines of evidence suggest that PPAR $\gamma$  ligands may be useful for the prevention of lung cancer. PPAR $\gamma$  ligands inhibit the growth of multiple histologic subtypes of lung cancer cell lines in vitro and in a xenograft animal model system, resulting in decreased proliferation, increased apoptosis, and induction of differentiation.<sup>141,142</sup> Furthermore, multiple animal carcinogenesis studies have demonstrated that PPAR $\gamma$  ligand treatment inhibits lung tumor development and can induce apoptosis.<sup>143,144</sup> Additionally, Wang et al<sup>144</sup> demonstrated that tumor inhibition occurs in the setting of p53 mutation as well, thereby mirroring the most common human genetic abnormality found in lung cancer, whereas Fu et al<sup>103</sup> showed that the addition of the PPAR $\gamma$  ligand, pioglitazone, to the inhaled steroid budesonide further improved the efficacy associated with either agent alone. Lung-specific PPAR $\gamma$ -overexpressing mice develop 70% fewer tumors than wild-type control mice when exposed to carcinogen.<sup>127</sup> Taken together, these studies suggest that PPAR $\gamma$  ligands may have the ability to affect the development of different histologic subtypes of lung cancer. Finally, epidemiologic analysis of a US Department of Veterans Affairs database demonstrated a 33% decrease in lung cancer in a population of male patients with diabetes taking thiazolidinediones compared with other antidiabetic agents,<sup>145</sup> although a large cohort study from the Kaiser Permanente Northern California Diabetes Registry failed to duplicate these results (hazard ratio, 0.9-1.0 for ever using pioglitazone or other thiazolidinediones).<sup>146</sup> Differences in cohorts, with less tobacco exposure highly likely in the Kaiser cohort, may be responsible for these discrepant results. Nevertheless, the cumulative evidence from these various types of studies provides the rationale for lung cancer prevention trials that are currently actively accruing participants. There is an ongoing US Department of Veterans Affairs-sponsored phase 2b trial of pioglitazone vs placebo for at-risk subjects, with endobronchial histology as the primary end point.<sup>147</sup>

### 6.3 *Mammalian Target of Rapamycin Inhibitors*

Mammalian target of rapamycin (mTOR) is a serine/threonine kinase that mediates the akt signaling

pathway. Human lung cancers, as well as dysplastic endobronchial lesions,<sup>148</sup> exhibit activation of the akt/mTOR pathway. Tobacco-specific carcinogens also activate this pathway,<sup>149,150</sup> and mTOR inhibitors (rapamycin and sirolimus) can successfully induce cell-cycle arrest. Early-phase clinical studies evaluating mTOR inhibitors (including metformin) are in the planning stage.

#### 6.4 Tea Extract

Many studies suggest that tea consumption protects against chronic diseases, such as cardiovascular disease and cancer. Tea contains high levels of flavonoids, which are phytochemicals thought to play key roles in preventing cancer. Tea has demonstrated significant antineoplastic effects in animal models of lung, skin, esophageal, and gastrointestinal cancers.<sup>151</sup> For example, green tea has been shown to inhibit lung tumor development in A/J mice treated with tobacco-specific carcinogens.<sup>152</sup> Green tea extract and epigallocatechin-3-gallate have also been shown to inhibit lung cancer in murine xenografts.<sup>153,154</sup> The potential of green tea extract for lung cancer chemoprevention is being evaluated in an ongoing phase 2 randomized controlled trial.<sup>155,156</sup>

Whereas the majority of the work evaluating the antineoplastic effects of tea has focused on green tea, the potential health benefits of white tea are becoming increasingly recognized. In one report, white tea extract was shown to be more efficacious than green tea extract in the induction of apoptosis in NSCLC cell lines, through the upregulation of 15-LOX, 15-hydroxyeicosatetraenoic acid, and PPAR $\gamma$ , supporting further evaluation of white tea extract for lung cancer chemoprevention.<sup>157</sup>

#### 6.5 Vitamin D

Studies have demonstrated that vitamin D in the form of 1,25 dihydroxycholecalciferol (or calcitriol) has significant antiproliferative activity in vitro and in vivo in a variety of murine and human tumor models including lung squamous cell carcinoma.<sup>158</sup> Calcitriol induces G<sub>0</sub>/G<sub>1</sub> arrest and modulates p27<sup>Kip1</sup> and p21<sup>Waf1/Cip1</sup>, the cyclin-dependent kinase inhibitors implicated in G<sub>1</sub> arrest.<sup>159</sup> The phosphorylation and expression of Akt, a kinase regulating a second cell survival pathway, is also inhibited after treatment with calcitriol. Pharmacokinetic studies have shown that the response to calcitriol administration is extremely variable among individuals, suggesting a genetic basis regulating metabolism. The ability of calcitriol to induce cell cycle arrest, apoptosis, and differentiation at doses without toxicity, makes calcitriol an attractive lung cancer chemopreventive agent. There is an ongoing phase 1 study in high-risk patients with

lung cancer evaluating the toxicity of calcitriol (a dose of 45  $\mu$ g every other week) in patients who do not have cancer.<sup>160</sup>

#### 6.6 Tyrosine Kinase Inhibitors

In lung carcinogenesis, the overexpression of EGFR affects the proliferative signaling pathways in the epithelium,<sup>161</sup> thereby increasing cell proliferation, cell motility, angiogenesis, and the expression of extracellular matrix proteins, and inhibiting apoptosis. The biology of EGFR is reviewed more extensively in the biology article. EGFR is also overexpressed consistently in premalignant lung lesions,<sup>162,163</sup> and expression is most pronounced in severe dysplasia and carcinoma in situ, offering a potential target for agents such as specific EGFR inhibitors. Increased expression ranged from 58% in low-grade lesions (squamous metaplasia and mild dysplasia), to 88% in high-grade lesions (moderate to severe dysplasia). These findings suggest that EGFR may also be a potentially important target for lung cancer chemoprevention trials.

Erlotinib is a human EGFR type 1/EGFR tyrosine kinase inhibitor. A phase 1 chemoprevention trial<sup>164</sup> is ongoing in high-risk subjects with lung cancer using a de-escalating dose of erlotinib (starting at 75 mg/d). The primary end point of this trial is the reduced ratio of active EGFR to total EGFR in endobronchial biopsy specimens.

##### 6.7.1 Recommendations

**6.7.1. For individuals at risk for lung cancer or with a history of lung cancer, the use of pioglitazone or myoinositol, for primary, secondary, or tertiary lung cancer chemoprevention is not recommended (outside of the setting of a well-designed clinical trial) (Grade 1B).**

**6.7.2. In individuals at risk for lung cancer, the use of tea extract, or metformin is not suggested for primary, secondary or tertiary prevention of lung cancer (outside of the setting of a well-designed clinical trial) (Grade 2C).**

#### 7.0 OTHER POTENTIAL TARGETED AGENTS FOR DEVELOPMENT

Many other targeted therapeutic agents exist with potential as chemopreventive agents. Some potential targets for lung chemoprevention, are:

- cyclooxygenase
- prostacyclin
- histone deacetylase
- insulin growth factor 1 receptor
- mammalian target of rapamycin
- epidermal growth factor receptor

- protein kinase C
- signal transducer and activator of transcription 3
- 5-, 12-lipoxygenase
- vascular endothelial growth factor

Selected targets are discussed here.

### 7.1 Protein Kinase C

Protein kinase C is involved in cellular proliferation, apoptosis, and mobility.<sup>165</sup> Enzastaurin, a protein kinase C- $\beta$  inhibitor, is being studied currently in patients with glioblastoma, lung cancer, and non-Hodgkin's lymphoma. The role of protein kinase C in carcinogenesis is complex, because there are 12 known isoforms with distinct effects. The  $\beta$  isoform is activated by growth factors, and enzastaurin competes with the ATP-binding site of protein kinase C- $\beta$ . In lung cancer cells, enzastaurin demonstrates inhibitory activity on intracellular signaling proteins,<sup>166,167</sup> and this drug is a possible candidate for chemoprevention in high-risk individuals. As demonstrated by the COX-2 inhibitor experience, extensive data on the safety and efficacy are needed prior to their application to the realm of chemoprevention.

### 7.2 Farnesyltransferase Inhibitors

Ras is an oncogene that is important for cancer cell survival. Farnesyltransferase inhibitors block ras farnesylation. Preclinical murine studies have shown farnesyltransferase inhibitors to be strong chemopreventive agents,<sup>168,169</sup> and this prompted further inves-

tigation in patients with lung cancer. The side effects experienced by participants in these trials included myelosuppression, nausea, diarrhea, abdominal pain, and fatigue. Its clinical toxicity limits this drug as a prime candidate for chemoprevention trials despite the promising preclinical data.

## 8.0 CONCLUSIONS/FUTURE DIRECTIONS

Lung cancer chemoprevention is a developing area of research whose main goal is to find an effective agent with a favorable toxicity profile for subjects at a high risk of developing a primary or secondary lung cancer. Lung cancer is no longer viewed as a single disease broken down into histologic categories; instead, genotyping of tumors to identify targetable molecular abnormalities for which there are approved therapies (eg, erlotinib for EGFR mutations and crizotinib for echinoderm microtubule-associated protein-like 4-anaplastic lymphoma kinase translocations) is becoming integrated into routine clinical practice prior to systemic treatment and is resulting in truly personalized therapy for a subset of patients with NSCLC. However, for several reasons, the same personalized approaches have yet to be applied to lung cancer prevention. Tobacco-induced lung carcinogenesis is intrinsically more complex than nonsmoking lung cancer, with the occurrence of many more mutations. The challenges of identifying the molecular drivers of carcinogenesis prior to the development of tumors that can be analyzed are self-evident. In this context, the

FIGURE 2. [Section 8.0] Results of large-scale chemoprevention trials in lung cancer.

Intervention	Study	Year	Endpoint	Number of Participants	Results
Aspirin	UK Physicians Health Study <sup>74</sup>	1988	Lung Cancer	5,139	Negative
	US Physicians Health Study <sup>75</sup>	1989	Lung Cancer	22,071	Negative
	US Womens' Health Study <sup>76</sup>	2005	Lung Cancer	39,876	Negative
Beta Carotene	ATBC <sup>65</sup>	1994	Lung Cancer	29,133	Harmful
	US Physicians Health Study <sup>67</sup>	1996	Lung Cancer	22,071	Negative
Beta Carotene & Retinyl Palmitate	CARET <sup>7</sup>	1996	Lung Cancer	18,314	Harmful
Retinyl Palmitate	Euroscan <sup>72</sup>	2000	2 <sup>nd</sup> Primary Lung Cancer	2,592	Negative
13-cis-retinoic acid	NCI Intergroup Trial <sup>73</sup>	2001	2 <sup>nd</sup> Primary Lung Cancer	1,166	Negative
Vitamin E	ATBC <sup>65</sup>	1994	Lung Cancer	29,133	Negative
N-Acetylcysteine	Euroscan <sup>72</sup>	2000	2 <sup>nd</sup> Primary Lung Cancer	2,592	Negative
Selenium	NCI Intergroup Trial <sup>85</sup>	2010	2 <sup>nd</sup> Primary Lung Cancer	1,772	Negative
	SELECT <sup>86</sup>	2011	Secondary Endpoint: Lung Cancer Incidence & mortality	35,533	Negative

ATBC =  $\alpha$ -Tocopherol  $\beta$ -Carotene study; NCI = National Cancer Institute; SELECT = Selenium and Vitamin E Cancer Prevention Trial. See Figure 1 footnote for expansion of other abbreviations.

approach described by Gustafson et al<sup>101</sup> to identify abnormal signaling pathways in the histologically normal epithelium of at-risk smokers offers new possibilities for personalized cancer prevention. Such high-throughput approaches have the potential to identify those at highest risk to target for interventions, to determine which interventions to offer based on the molecular profile of the individual, and to follow the effectiveness of the interventions.

Despite a rapidly increasing understanding of the mechanisms of lung carcinogenesis, the prevention of lung cancer has proven to be complicated. To date, no phase 3 chemoprevention trial has shown benefit, and some trials have shown harm. Fig 2 summarizes the large phase 3 trials that have been conducted. Although the phase 3 trials were disappointing, they did provide useful lessons that continue to shape the design of current trials, including the importance of taking environmental (as well as host) factors into consideration when conducting chemoprevention trials. These large trials have underscored the fact that small increases in adverse effects cannot be appreciated without large and lengthy clinical trials, even though such small increases may have a large public health impact, given the number of people at risk. Finally, these trials have reinforced the lesson that nutritional supplements, like other pharmacologic interventions, can have significant negative side effects and therefore these agents must also be tested in rigorous clinical trials.

The research emphasis has therefore shifted to phase 2 studies, which are intended to demonstrate preliminary efficacy and additional evidence of drug effects on multiple potential intermediate end points as well as toxicity. In addition, several smaller phase 2 chemoprevention trials have been performed using histologic criteria, such as metaplasia and dysplasia in endobronchial biopsy specimens, cellular atypia in cytologic sputum specimens, and Ki-67 labeling index, as intermediate end points. Examples of agents that have been investigated include various retinoids,<sup>170-173</sup> folate and vitamin B12,<sup>174</sup> and budesonide,<sup>135</sup> but only oral iloprost has shown improvement in endobronchial dysplasia.<sup>128</sup> Areas of active research include the identification of the highest-risk cohorts who stand to benefit the most from preventive interventions, the identification of appropriate targets for which safe and effective interventions exist or can be designed, the identification of intermediate (surrogate) end points, and the initiation of new clinical trial models to allow more efficient evaluation of candidate interventions.

The future of lung cancer chemoprevention should entail the evaluation of single drugs or drug combinations (with supportive preclinical data) that will target multiple pathways while working toward identifica-

tion and validation of intermediate end points. Despite this promising future, to date no one agent is recommended for use in the chemoprevention of lung cancer. It is strongly recommended that individuals at high risk of lung cancer or with a history of lung cancer be encouraged to participate in lung cancer chemoprevention trials.

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*Dr Szabo:* contributed to the conception and design; collection and assembly of data; data analysis and interpretation; manuscript writing, review, and revision; and final approval.

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**Additional information:** The supplement table can be found in the "Supplemental Materials" area of the online article.

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