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Premalignant Progression in the Lung: Knowledge Gaps and Novel Opportunities for Interception of Non–Small Cell Lung Cancer

An Official American Thoracic Society Research Statement

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Abstract

Rationale: Despite significant advances in precision treatments and immunotherapy, lung cancer is the most common cause of cancer death worldwide. To reduce incidence and improve survival rates, a deeper understanding of lung premalignancy and the multistep process of tumorigenesis is essential, allowing timely and effective intervention before cancer development.

Objectives: To summarize existing information, identify knowledge gaps, formulate research questions, prioritize potential research topics, and propose strategies for future investigations into the premalignant progression in the lung.

Methods: An international multidisciplinary team of basic, translational, and clinical scientists reviewed available data to develop and refine research questions pertaining to the transformation of premalignant lung lesions to advanced lung cancer.

Results: This research statement identifies significant gaps in knowledge and proposes potential research questions aimed at expanding our understanding of the mechanisms underlying the progression of premalignant lung lesions to lung cancer in an effort to explore potential innovative modalities to intercept lung cancer at its nascent stages.

Conclusions: The identified gaps in knowledge about the biological mechanisms of premalignant progression in the lung, together with ongoing challenges in screening, detection, and early intervention, highlight the critical need to prioritize research in this domain. Such focused investigations are essential to devise effective preventive strategies that may ultimately decrease lung cancer incidence and improve patient outcomes.

Keywords: lung cancer; premalignant progression; oncogenic transformation; interception

Overview

Premalignant progression in the lung represents a critical phase in the development of non–small cell lung cancer (NSCLC), offering a vital window for early interception and prevention. Despite advances in understanding the molecular and cellular pathways driving lung carcinogenesis, significant knowledge gaps persist regarding the precise mechanisms underlying premalignant progression and the identification of effective interception strategies. Lung premalignant progression is influenced by a range of factors, including cellular origins, chronic lung diseases, environmental exposures, DNA damage, cellular stress, and metabolism. It also involves intricate cross-talk among diverse cell populations within the lung microenvironment, such as innate

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inflammatory responses in the airway epithelium and cell-mediated immune surveillance mechanisms. Despite significant advances in our understanding of the molecular and cellular pathways driving lung carcinogenesis, substantial knowledge gaps remain regarding the precise mechanisms underlying premalignant progression and effective strategies for its interception. This statement summarizes our current understanding of the etiology and biology of premalignant airway transformation, highlighting identified gaps in knowledge. Our discussion extends to preclinical modeling approaches crucial for deciphering the early phases of lung carcinogenesis. In addition, we examine risk assessment approaches, biomarker identification, and chemoprevention strategies, which are pivotal elements in early detection, prognostication, and intervention.

The key topics and knowledge gaps discussed include the following:

- underlying molecular mechanisms of oncogenic transformation in response to injury associated with early metabolic alterations, epigenetic changes, and genomic instability;
- the key role of lung inflammation and host precancer immune/stromal interaction in facilitating tumor development and progression; and
- new strategies and opportunities to intercept progression of premalignant disease.

Collectively, by identifying these knowledge gaps and exploring emerging opportunities, we have complied a list of potential research questions; addressing these questions could lead to the development of early detection methods and targeted strategies for intercepting NSCLC at its earliest stages as well as prognostic markers that could ultimately improve patient outcomes and reduce the burden of this devastating disease.

Introduction

Lung cancer remains the second most prevalent cancer and the leading cause of cancer-related death globally, with NSCLC representing the majority of cases (1, 2). NSCLC is primarily categorized into two subtypes: lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC), each originating from distinct progenitor cells and progressing through unique trajectories through histologically well-defined premalignant stages (3–6). Despite advances in the development of targeted therapies and immunotherapies for advanced-stage NSCLC, primary and acquired therapeutic resistance persists. Enhancing our understanding of cellular transformations occurring during the premalignant phase, before the manifestation of overt carcinoma, is essential for enabling early detection and intercepting disease progression (7, 8).

In this research statement, we examine the biological underpinning of premalignant progression in the lung, identify prevailing deficits in knowledge, and explore innovative interception strategies. Our main objective is to delineate the intricate interplay of biological, environmental, and microenvironmental factors that propel the evolution of preneoplastic lesions and their progression to advanced NSCLC. We evaluate the challenges associated with early detection and risk stratification, explore the potential advances in detecting high-risk individuals, and introduce possible targeted interception strategies.

Collectively, this comprehensive research statement aims to bridge the existing knowledge gaps, provides insights into lung premalignancy, and defines promising avenues for intercepting NSCLC while laying the foundation for identifying biomarkers and new targets. Our overarching goal is to guide future research and clinical interventions focused on modifying the course of premalignant lung disease to halt the development of lung cancer.

Methods

This project of the American Thoracic Society (ATS) Assembly on Thoracic Oncology was reviewed and approved by the ATS Program Review Subcommittee. A diverse international multidisciplinary panel was assembled by the chairs (S.J.M., R.S., and R.L.K.), including pulmonologists and basic, translational, and clinical scientists with expertise in lung cancer cellular and molecular biology, immunology, pathology, screening, diagnosis, early detection, prevention, treatment, and clinical trials. Conflicts of interest were disclosed and managed according to ATS policies and procedures.

The chairs initially developed an overview of the project goals and objectives, which was shared with all the panel members. These objectives were further defined during a general conference call with the expert panel. Three subgroups were established, each led by a designated leader (C.F.K., A.M.H., and R.L.K.). Panel members were assigned or selected to join subgroups on the basis of their expertise and areas of interest, as outlined in Table 1. Each subgroup then convened to further delineate the subaims and distributed tasks involving the collection of current knowledge, identification of knowledge gaps, formation of research questions, and development of potential approaches to address these questions. Each subgroup collaborated to prepare a written draft for its respective objectives.

Table 1. Committee Members and Their Roles

Tasks	Participants
Co-chairs	Seyed Javad Moghaddam Rajkumar Savai Robert L. Keith
Objective 1	Carla F. Kim Catherine R. Sears Carmen Priolo Alison K. Bauer Jennifer E. Beane Shreoshi Sengupta Rajkumar Savai Meredith A. Tennis Humam Kadara Ramin Salehi-Rad Seyed Javad Moghaddam
Objective 2	Ramin Salehi-Rad Brendan J. Jenkins Steven M. Dubinett A. McGarry Houghton Rajkumar Savai Seyed Javad Moghaddam
Objective 3	Michael N. Kammer Edwin J. Ostrin Meredith A. Tennis Laura P. Stabile Eva Szabo M. Patricia Rivera Charles A. Powell Humam Kadara Pierre Massion Robert L. Keith

In first objective, we investigated the complex biological mechanisms that orchestrate oncogenic transformation. This included exploring early genomic alterations triggered by various insults to unravel the molecular complexities that drive normal cells toward malignancy. The second objective focused on the intricate interplay between the lung inflammatory milieu and host immune responses with epithelial and/or tumor cells, aiming to elucidate the dynamic landscape that shapes the transition from precancerous states to established tumors. Finally, the third objective delved deeper into premalignant lesion (PML) biology and preclinical modeling of LUAD and LUSC, proposing innovative strategies and opportunities for the detection, prediction, and interception of premalignant diseases. The drafts from each subgroup were reviewed and finalized by the subgroup leaders and chairs. The chairs compiled all three drafts and prepared a final manuscript, which was shared with all the panel members for their final inputs before undergoing peer review and receiving final approval by the ATS Board of Directors.

Results

To achieve our goals, we conducted a comprehensive exploration of three objectives, determined important research subtopics in each objective, came up with related knowledge gaps and research questions, and accordingly proposed potential strategies to address these gaps.

Objective 1: Identification of the Underlying Molecular Mechanisms of Oncogenic Transformation in Response to Injury Associated with Early Metabolic Alterations, Epigenetic Changes, and Genomic Instability

Advanced-stage lung cancers exhibit significant molecular heterogeneity, with most treatment strategies leading to the development of resistance. The two most common types of NSCLC, LUAD and LUSC, each have different cells of origin and develop through a series of histologically defined premalignant stages (3–6) that precede invasive cancer. A thorough investigation of these premalignant stages, focusing on the conditions that drive tumorigenesis, including predisposing lung injury, lung disease, and environmental exposures, will identify metabolic drivers, DNA damage, and altered cellular states that drive progression (Figure 1). These aspects are discussed in further detail in this objective.

Premalignant airway biology and preclinical modeling. LUSC. LUSC originates from the bronchial epithelium and progresses through defined stages of premalignancy to invasive carcinoma. During progression, the ciliated glandular epithelium undergoes morphologic changes to form a squamous epithelium characterized by increasing cellular disarray at the basal membrane and cytologic atypia (9). PMLs are commonly found in patients exposed to cigarette smoke or other inhaled toxicants. Although high-grade lesions and carcinoma *in situ* have the highest risk of progressing to invasive LUSC, even the earliest lesions can have genetic abnormalities that make them at risk for progression (10). Identifying at-risk PMLs is a major challenge in LUSC, as lesions of all PML stages can remain stable for years or even regress to a lower grade or a normal phenotype (11). High-grade or progressive PMLs are associated with an immunosuppressive environment, which may be supported by alterations in YAP and TAZ gene regulation of TP63, transcriptional enhanced associated domains, and MHC2TA (12–14). PMLs that progress commonly have mutations in TP53, CDKN2A, SOX2, and AKT2, whereas those that fail to progress have a lower mutational burden (15). Genomic instability likely affects epithelial biology in the early phases of squamous carcinogenesis, on the basis of the presence of mutational signatures associated with smoke exposure and altered deaminase activity (16). PMLs acquire increasing epigenetic modifications through progressive stages, including methylation of RARB, FHIT, MGMT, RASSF1, DAPK1, APC, and CDH1, with potentially hundreds of methylated genes observed in carcinoma *in situ* lesions (15, 17). Alterations in microRNA during squamous PML progression have not been fully characterized, but on the basis of a limited study of bronchial biopsies and studies in carcinogen-exposed human bronchial epithelial cells in culture, they likely contribute significantly to the initiation and progression of PMLs (18, 19). Numerous studies have identified

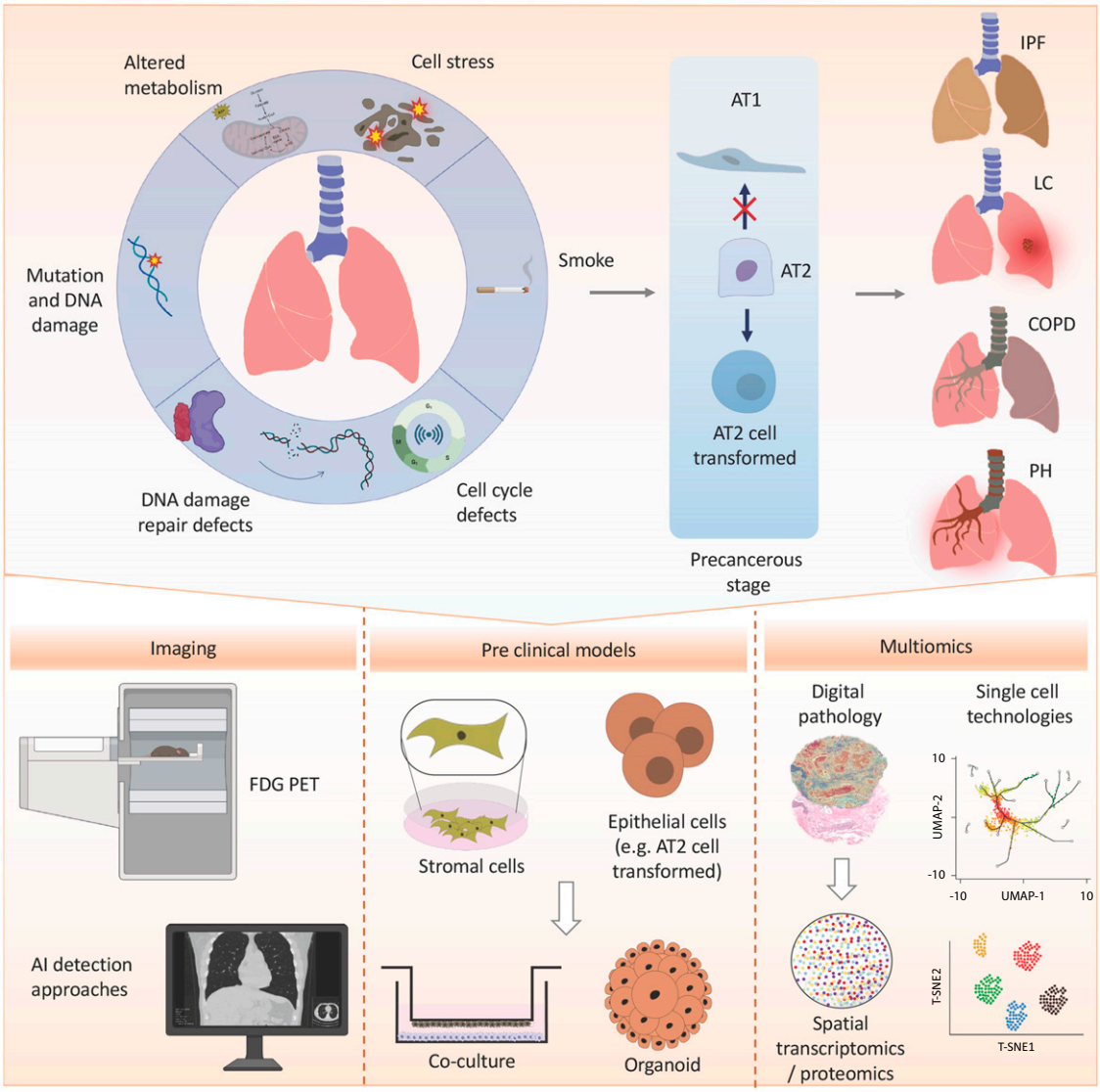

Figure 1. Underlying molecular mechanisms of oncogenic transformation, knowledge gaps, and potential tools for addressing them. Environmental exposures, mutations, DNA damage, cellular stress, and altered metabolism serve as pivotal triggers that compromise the function of alveolar type II (AT2) epithelial cells in response to lung injury. The malfunction in AT2 cell regeneration leads them into a transcriptional transition state, evolving into highly adaptable cells during the precancerous phase. Age-related AT2 dysfunction disrupts repair and regenerative processes, exacerbating lung diseases such as COPD, IPF, PH, and LC. Cutting-edge diagnostic tools such as PET scans play a crucial role in the early detection and visualization of premalignant lesions. These scans, coupled with comprehensive omics analyses, elucidate the molecular alterations underlying neoplastic transformations in the initial stages of tumorigenesis. Furthermore, *ex vivo* model systems, such as cocultures and organoid cultures involving stromal and epithelial cells, offer invaluable insights into the influence of the microenvironment across various stages of carcinogenesis. Complementary techniques such as digital pathology, single-cell analysis, and spatial multiomics approaches are used to investigate early clonal progression and intercellular dynamics within the tumor microenvironment. This integrated approach provides a comprehensive understanding of cellular dynamics and molecular pathways in both premalignant and malignant lesions. AI = artificial intelligence; AT1 = alveolar type I; COPD = chronic obstructive pulmonary disease; FDG = [^{18}F]2-deoxy-2-fluoro-D-glucose; IPF = idiopathic pulmonary fibrosis; LC = lung cancer; PET = positron emission tomography; PH = pulmonary hypertension; T-SNE = t-distributed stochastic neighbor embedding; UMAP = uniform manifold approximation and projection.

pathway changes critical to PML biology, such as proliferation, cell adhesion, extracellular matrix organization, cell cycle regulation, DNA repair, and antigen presentation (11).

Multiomics characterization of patient samples at different stages of premalignancy has fueled the development of preclinical models to more closely mimic human pathology. Unlike LUAD, preinvasive LUSC has proved challenging to model in mice. Although there have been major advances in models that recapitulate the preinvasive stages of LUSC development, there remains an urgent need for further refinement of

current models and the development of new models to facilitate the testing of early intervention strategies. Continuous efforts are being made to generate clinically relevant genetically engineered mouse LUSC models (20). However, these are limited by the lack of complex mutational heterogeneity present in human LUSC (21, 22). Although chemical carcinogen–induced models are the most widely used for interception studies, they bring challenges such as long latency periods, toxicity, mixed histologies, strain- and sex-dependent effects, and generally low tumor yield. Currently, the best characterized LUSC model used for chemoprevention studies is the N-nitroso-tris-chloroethylurea (NTCU) mouse model. When applied topically, NTCU induces epithelial histologic changes that mimic the stepwise evolution of human LUSC (23–25), and resulting tumors have genomic (26, 27) and transcriptomic (28) similarity to those in patients. The addition of cigarette smoke to the NTCU regimen has been shown to reduce toxicity and shorten the time to PML development, but whether it better represents human disease remains to be determined (29). *In vitro* modeling, including air–liquid interface cultures, autologous cocultures, and organoid cultures from human or mouse bronchial epithelial cells or patient-derived cultures remain valuable tools to dissect LUSC molecular drivers and vulnerabilities, but they fail to fully recreate the complex cell–cell interactions in the lung. *Ex vivo* precision-cut lung slice models have been increasingly used to overcome the limitations of *in vitro* models but do not provide access to the wider, recruitable immune system. The lack of good preclinical PML models has hampered the translation of prevention agents to the clinic. It is critical that the same characterization performed on human tissue also be done on mouse tissue to validate these models. Future research should focus on an improved understanding of the precancer microenvironment and immune cell interplay throughout disease progression, as stated in objective 2.

The main research questions in this area that need to be answered are as follows: *I-1*) Will models of LUSC premalignancy recapitulate genetic alteration and tumor microenvironment (TME) changes observed in individuals with smoking history? and *I-2*) If not, how can the model be improved?

LUAD. Earlier work showed that a widespread field of injury (also termed field carcinogenesis) exists in the form of molecular, genetic, and inflammatory changes in the lung (30–38). Many alterations in normal-appearing tissue (NAT) are enriched in the local and epithelial niche of LUADs but not in relatively more distant normal-appearing regions (39–40), suggestive of changes that may underlie the transition from NAT to LUAD. Members of this working group and others, mostly using imaging and bulk sequencing approaches, identified molecular and immunologic trajectories along the spectrum of NAT to adenomatous PMLs (aPMLs; precursors for LUAD) and up to LUADs (41–44). Notably, *KRAS* was the most frequently mutated oncodriver in NAT and aPMLs (40, 44). Interestingly, *BRAF* and *KRAS* mutations in LUAD precursors were mutually exclusive and differentially enriched in individuals with and without smoking history, respectively. Of note, unlike *KRAS* and *EGFR* variants, *BRAF* mutations in LUAD precursors were rarely found in matching LUAD counterparts, suggesting bimodal fates in the PML–LUAD trajectory that are dependent largely on the initiating mutation (i.e., *BRAF* vs. *KRAS*) (44). These observations recapitulate earlier studies in mice where *BRAF*-mutant lung adenomas rarely, if ever, progressed to invasive LUADs *in vivo* (45). Krysan and colleagues mapped the mutational and neoantigen landscape of PMLs and LUADs (42) and found that although oncodriver mutations were more frequently present in LUADs compared with PMLs, the latter still had abundant enrichment for mutational signatures related to DNA damage repair (42). Other studies have demonstrated somatic point mutations, copy-number alterations, allelic imbalance, methylation profiles, immunogenomic changes, and spatial changes in the organization of immune niches along the pathological spectrum of the normal lung to aPML to LUAD (41–44, 46).

With the advent of single-cell sequencing technology and, more recently, spatial omics platforms, new knowledge has emerged on the biology of cancer ecosystems (47, 48). However, their application to study aPMLs remains challenging because of inherent roadblocks related to longitudinal sampling during the transition from NAT to LUAD. Atypical alveolar hyperplasia (AAH) is the only recognized preneoplastic lesion for LUAD. The World Health Organization and the International Association for the Study of Lung Cancer have subclassified LUAD on the basis of predominant cell morphology and growth patterns, such as adenocarcinoma *in situ* (AIS), minimally invasive adenocarcinoma (MIA), lepidic predominant adenocarcinoma that contain an invasive component, adenocarcinoma with mixed subtypes, and homogeneously invasive solid adenocarcinoma with a variety of invasive histologic patterns. The key histologic distinction among these subtypes of LUADs is tissue invasion, a cancer hallmark as the first step of the metastasis process, and a precursor of an activated tissue microenvironment characterized by functional alterations of fibroblasts, endothelial proliferation, and immune dysregulation. The spectrum of intratumoral histologic heterogeneity in LUAD suggests that invasiveness represents a continuum of disease, from noninvasive AIS to lepidic predominant adenocarcinoma to solid invasive adenocarcinoma (49, 50) that can lead to metastasis and tumor dissemination. Thus, although AIS is accepted as a preinvasive lesion, consistent identification and differentiation from very early locally invasive LUADs such as MIA depend on rigorous pathological review and annotation (34). PMLs are very rarely, if ever, surgically resected (8, 51). Their location deep in the lung periphery and thus relative inaccessibility, together with their very small size, makes them extremely difficult to biopsy and sample longitudinally. PMLs of LUADs are almost always archived from surgical tumor resection specimens, and their small size and near universal processing as formalin-fixed, paraffin-embedded (FFPE) specimens, render them very difficult to characterize with multimodal single-cell approaches.

The specific knowledge gaps and main research questions are as follows: *I-3*) What are the states and properties of specific cell subsets, and how do they interact in space in conjunction with anatomical features of lung tissue that underlie transitions along the normal lung–aPML–LUAD spectrum?

Cellular origins, chronic lung disease, and early tumorigenesis. Lung repair is performed by different stem or progenitor cell populations distributed along the complex lung environment. Among the best characterized progenitor cells are alveolar type II epithelial (AT2) cells (52, 53). AT2 cells facilitate gas exchange and maintain tissue homeostasis. However, it is not clear how these progenitor cells are affected at a molecular level by age, environmental

influences, or infections and how these affect disease susceptibility. New findings suggest that LUAD arises from AT2 cells (53, 54); thus, AT2 cell dysfunction in various conditions likely contributes to tumorigenesis. When AT2 cells respond to lung injury in the form of alveolar damage, they enter a temporary transcriptional cell state, referred to as a "transitional state," characterized by plasticity. Subsequently, these cells give rise to alveolar type I cells. This transitional cell state has been identified in RNA sequencing analysis of pulmonary fibrosis samples and, more recently, has been shown to persist in both murine and human lung tissues in association with oncogenic *KRAS* mutations and the development of LUAD (55, 56). In mouse and human lung tumors, a cell state with high plasticity that shares similarity with the aforementioned transitional cell state drives cellular heterogeneity and drug resistance and is associated with poor patient prognosis (57). Lineage switching of *Kras* mutant LUAD to LUSC through the deletion of *Lkb1* has been observed in autochthonous and transplant models, rendering targeted therapy challenging and often futile (58). In addition, transformation of EGFR-mutated LUADs into an aggressive neuroendocrine lineage, small cell lung cancer, has been detected after treatment with EGFR inhibitors (59). Plasticity-driven expansion of spatially localized subclones of tumor cells induces critical processes such as metastasis (60). Although ongoing research is revealing the critical role of plasticity in tumor aggressiveness and treatment resistance, much is unknown about the impact of cell plasticity on the development of lung cancer.

Key questions that remain to be answered include the following: *I-4*) How do lung epithelial cells (e.g., AT2 cells) acquire a plastic nature that drives LUAD initiation? *I-5*) What factors within the normal and premalignant microenvironment drive plasticity in the early stages of carcinogenesis? and *I-6*) How does this affect disease aggressiveness?

Pulmonary arterial hypertension, chronic obstructive pulmonary disease (COPD), and idiopathic pulmonary fibrosis (IPF) are heterogeneous chronic lung diseases characterized by structural changes in the lung parenchyma and airway vasculature. These changes compromise airway barrier integrity and lung regenerative programs, leading to an increased risk of lung cancer and/or a poorer prognosis (61, 62). Chronic inflammation, impaired wound-healing responses, and dysregulated tissue repair mechanisms are associated with the development of these lung diseases. Age-related dysfunction of lung progenitor cells impairs the ability to repair and regenerate, contributing to lung diseases such as COPD and IPF (63). Age-related changes in the frequency of various progenitor populations in the lung have also been described, but the study of their functional characteristics and how this leads to lung disease is limited (64–66). In addition, genetic factors can contribute to the development of lung cancer, IPF, and COPD. Understanding the common and distinct processes underlying the transition from COPD, IPF, and pulmonary hypertension to lung cancer requires a comprehensive approach that overcomes the challenges of the lung microenvironment and disease heterogeneity.

Key gaps and, thus, opportunities for research in the field of lung cancer comorbidities include the following: *I-7*) What are the roles of epithelial injury, aging, and resilience in phenotypic plasticity, defective regeneration, and profibrotic signaling of the alveolar epithelium to the premalignant microenvironment? *I-8*) What structural changes and alterations in cell–cell communication in the lung parenchyma contribute to the development and progression of lung cancer in patients with COPD, IPF, and pulmonary hypertension compared with patients without these comorbidities? and *I-9*) How do these changes in the lung parenchyma of patients with COPD or IPF lead to altered cell states within AT2 and other epithelial cells?

DNA damage and cellular stress. Lung cancers exhibit high degrees of DNA damage and genomic instability caused by both exogenous sources (e.g., inhalation of carcinogens, including direct or passive cigarette smoke, environmental pollution, asbestos, occupational exposure) and endogenous sources (indirect DNA damage caused by oxidative stress, inflammation, and cell metabolism) (67). DNA damage leads to cellular replication stress and a shift to low-fidelity DNA repair processes, ultimately resulting in genomic instability that characterizes lung neoplasia (68, 69). The presence of distinct patterns of genomic changes associated with the types of exposure and DNA repair status indicates that the accumulation of mutations is not random (70, 71). Impaired DNA damage repair also plays a critical role in early lung carcinogenesis, leading to genomic instability, which in turn accelerates the acquisition of hallmark biological traits associated with cancer (72). These traits include sustained proliferative signaling, apoptosis resistance, replicative immortality, metabolomic reprograming, induction of angiogenesis, and capacity for invasion and metastasis (67, 72). Studies have revealed that reduction of nucleotide excision and base excision repair pathways is strongly associated with increased prevalence of lung cancer (73, 74). DNA repair capacity may decrease with cigarette smoking and e-cigarette vape exposure, as well as with advancing age (75–77). Decreased DNA repair capacity has also been observed in patients with lung cancer who have never smoked (78, 79). In addition, mounting evidence suggests a causal relationship between inflammation and oxidative stress, resulting in altered DNA damage repair and lung tumor development (74, 78, 80–83) (discussed in further detail in objective 2).

However, our mechanistic understanding of the impact of DNA damage and impairments in DNA repair on the earliest events in lung carcinogenesis still has crucial gaps. Unanswered questions remain: *I-10*) How do oncogenic changes affect DNA repair capacity? and *I-11*) Which immune cells are affected by the response to DNA damage in combination with mutations?

Environmental exposures and emerging threats. Air pollutants increase the risk of developing lung cancer (84), especially LUAD. The International Agency for Research on Cancer classifies outdoor air pollution as carcinogenic (85). Particulate matter (PM) is a component of air pollution, and in a recent study, exposure to PM with an aerodynamic diameter $\leqslant$2.5 μm ($PM_{2.5}$) was found to increase the risk of EGFR-mutant LUAD (86). A large, combined cohort study identified a significant association between $PM_{2.5}$ concentrations and the locations where LUAD patients with *EGFR* mutations resided. A significant increase in the number of preinvasive neoplasms was observed in EGFR-mutant mice treated oropharyngeally with a standard reference material, SRM2786, a PM with a mean particle diameter of 2.8 μm, compared with those treated with saline (86). The number of hyperplasias in Kras-mutant mice was also increased in SRM2786-exposed mice, suggesting that PM can promote

tumor formation in both EGFR- and KRAS-driven murine models of LUAD. In addition, exposure of $EpCAM^+$ cells from EGFR-mutant mice to SRM2786 produced significantly more organoids than those from nonmutant mice and triggered a change in AT2 cells to a more progenitor-like transcriptional state compared with control animals (86). The mechanism inducing the changes in the state of AT2 cells is likely related to the observed influx of macrophages and the release of cytokines such as IL-1, GM-CSF, CCL6, and IL-33. IL-1, for example, can mediate alveolar regeneration (87). In support of this hypothesis, a human clinical crossover study using bronchial brushes from former smokers with COPD and healthy control subjects exposed to diesel exhaust or filtered air found that former smokers with COPD may be susceptible to an acute inflammatory response after exposure to diesel exhaust (88). Because climate change can lead to increased air pollution, such as increased $PM_{2.5}$ generation from forest fires, further studies are needed to understand the mechanisms by which PM-induced molecular changes occur in lung epithelial cells and how they trigger PMLs and tumors.

Other environmental (indoor and outdoor) and/or occupational exposures that may influence lung tumorigenesis include nanomaterials, nanoplastics and microplastics, burn pits, e-cigarettes, and combustible cannabis (e.g., dabbing) (89–92). A study evaluating single-walled carbon nanotubes, an engineered nanomaterial commonly used in electronics, including transistors, storage devices, and batteries, can drive carcinogenesis in lung progenitors cells through the upregulation of SOX-9, a transcription factor critical for embryonic development and stem cell maintenance (91). In addition, e-cigarette extracts and nicotine have been found to induce the expression of embryonic stem cell factor SOX-2, another transcription factor critical for the maintenance of self-renewal and maintenance of stem cell properties in NSCLC cells (93). This induction leads to upregulation of epithelial-to-mesenchymal transition markers (vimentin, fibronectin, Zeb1, and Zeb2) and enhanced migration of NSCLC cells (93). Certain polycyclic aromatic hydrocarbons (e.g., fluoranthene) in air pollution have also been shown to inhibit gap junctions in AT2 cells (27, 94, 95), leading to uncontrolled proliferation (96).

Many important questions remain unanswered: *I-12*) What are the specific combinations of pollutants and cumulative doses that influence the development of lung cancer? *I-13*) How do different exposures affect lung progenitor cells, their plasticity, and their ability to promote PMLs and lung cancer? Although not discussed in the context of this section, underserved populations are often at a higher risk of adverse health effects, including cancer, which results in the following research questions: *I-14*) How do the social and behavioral determinants of health affect these environmental exposures that have the potential to lead to lung cancer? and *I-15*) What mitigation strategies can be used as prevention in these vulnerable communities?

Diversity and heterogeneity of tumor and stromal cells. Lung tumors exhibit heterogeneous phenotypes, both within (intra) and between (inter) tumors (97). Mutations, together with nonmutational processes such as alterations in the epigenetic programing of tumor and stromal cells, as well as changes in the signaling molecules and metabolic conditions of the TME, can drive molecular and phenotypic transformations leading to increased plasticity and heterogeneity of tumor and stromal cells (98–100). Intratumor heterogeneity within lung cancers can drive neoplastic progression and treatment resistance (60, 101). Pan-cancer analyses have demonstrated a strong association between intratumor heterogeneity and decreased survival in patients with various malignancies, including NSCLC (21, 102). Moreover, a decrease in clonal neoantigen burden in patients with NSCLC has been correlated with poor responses to immune checkpoint blockade (ICB), underscoring the impact of intratumor heterogeneity on host immune surveillance (103, 104).

Recent technological advances have enabled the in-depth characterization of tumor and stromal cells at a single-cell level. Single-cell RNA sequencing of early-stage lung tumors has confirmed significant heterogeneity in cellular composition, intercellular signaling networks, and developmental trajectory of cancer cells, together with molecular and cellular reprogramming of cancer-associated fibroblasts and tumor-infiltrating immune cells (60, 105–109). In addition, studies have emphasized significant variations in the expression of epigenetic regulators, such as DNA methyltransferases, histone deacetylases, microRNAs, and long noncoding RNAs, contributing to heterogeneity (107, 110–112). Evaluation of FFPE lung cancers with multiplex immunohistochemistry techniques, spanning chromogenic, metal-based, fluorescence-based, and DNA barcoding–based methods revealed considerable spatial heterogeneity of the stromal and immune landscape of the TME in lung cancer (42, 113, 114). Furthermore, significant diversity in lung cancer metabolism has been identified, strongly influencing the cellular phenotypes in the TME (115, 116). Although these studies have begun to delineate the spatial and temporal heterogeneity of lung tumors, our understanding of the cellular plasticity and genetic heterogeneity of lung neoplasia and their impact on tumorigenesis remains limited.

Key research questions in this area include the following: *I-16*) How does the spatiotemporal interplay among different stromal and immune cell types influence preneoplasia progression? and *I-17*) What influence do epigenome and epigenetic heterogeneity have on the TME of preneoplasia?

Metabolism and mitochondrial dysfunction. Metabolic derangements resulting from mitochondrial abnormalities occur in lung injury, chronic lung disease, and lung cancer. Because of elevated metabolic demand, AT2 cells have the largest number of mitochondria in the lung and can undergo active metabolic reprogramming, including increased use of lactate (117). Interestingly, primary mouse AT2 cells can maintain bioenergetic homeostasis and ATP concentrations under hypoxic conditions, comparable with cells in normoxia, without significantly increasing lactate concentrations (118). An accumulation of dysmorphic and dysfunctional mitochondria together with low expression of PINK1 (PTEN-induced putative kinase 1) have been described in AT2 cells of patients with IPF, who may typically experience hypoxia (119). In addition, PINK1-deficient mice develop similarly dysmorphic and dysfunctional mitochondria in AT2 cells and are prone to the development of pulmonary fibrosis (119). Deregulation of the mitochondrial fusion and fission machinery with increased expression of DRP-1 (dynamin-related protein 1), which promotes fission, has been described in human lung cancers (120).

Increased DRP-1 expression leads to excessively fragmented and metabolically dysfunctional mitochondria, resulting in increased cancer cell proliferation (120, 121). Somatic mitochondrial DNA mutations, including homoplasmic mutations, have been described in both lung cancer (122, 123) and IPF (124), with no found association with smoking status. These mitochondrial defects may be associated with metabolic reprogramming, including a prominent compensatory role for glucose metabolism. Moreover, enhanced glycolysis may be a primary metabolic event in lung tumorigenesis, triggered by common genetic drivers such as RAS and EGFR mutations. Interestingly, in $CD166^{+}$ lung tumor-initiating cells isolated from primary NSCLC tumors, a glycine decarboxylase–dependent metabolic program was described that enhances both glycine and serine metabolism and glycolysis to support pyrimidine synthesis. This study demonstrated that glycine decarboxylase metabolic function is required for tumor-initiating cell growth and tumorigenesis (125).

Recent studies using voltage-sensitive positron emission tomography (PET) traces, which profile the mitochondrial membrane potential of tumors *in vivo*, have shown significant metabolic heterogeneity in NSCLC with presence of functionally distinct metabolic and bioenergetic phenotypes for LUAD and LUSC tumors (126, 127). Although integrated computed tomography (CT) scans with [^{18}F]2-deoxy-2-fluoro-D-glucose (FDG) PET imaging have been found to be superior to PET or CT alone for the identification and staging of NSCLCs (128–130), this approach may not be effective for assessment of early lesions. This is partly because the GLUT transporters are not active in lung premalignancy. A recent study identified selective expression of SGLT (sodium-dependent glucose transporter 2) in lung premalignancy and early-stage LUADs. Selective targeting of SGLT2 with small-molecule inhibitors significantly reduced tumor growth and prolonged survival in murine models and patient-derived xenografts (131). SGLT2 activity can be detected *in vivo* by PET with the specific tracer methyl 4-deoxy-4-[^{18}F]-fluoro-α-d-glucopyranoside. An ongoing phase I and II clinical trial is evaluating the safety and efficacy of PET imaging with methyl 4-deoxy-4-[^{18}F]-fluoro-α-d-glucopyranoside for the early diagnosis of lung cancer. The early detection of lung cancer by PET imaging is also limited by the spatial resolution of PET systems (minimum diameter 0.7–0.8 cm). Technological advances, including the application of artificial intelligence approaches, may improve detection and image analysis. One promising avenue is the application of PET radiomics, which focuses on defining the "texture" of lesions (heterogeneity of signal) rather than the overall uptake of radiotracer (standardized uptake value) (132). The integration of PET radiomics and artificial intelligence technologies with spatial omics, including spatial transcriptomics and metabolomics (133), is necessary to facilitate the identification of metabolic fingerprints and biomarkers at the earliest stages of lung cancer development (134), paving the way for new PET radiotracers for the early detection.

Key gaps and research questions in these areas include the following: *I-18*) What are the metabolic features and metabolic dependencies of premalignancy and lung cancer, and at what stage of lung carcinogenesis does mitochondrial dysfunction occur? *I-19*) What are the causes and consequences of mitochondrial network deregulation in premalignant and cancer cells of the lung? and *I-20*) Can FDG PET detect PMLs of the lung that will develop into lung cancer?

Objective 2: Defining the Key Role of Lung Inflammation and Host Precancer Immune/Stromal Interaction in Facilitating Tumor Development and Progression

Lung tumorigenesis entails an intricate and dynamic interaction between preneoplastic cells and the surrounding TME, comprising the extracellular matrix, vasculature, lymphatics, cancer-associated fibroblasts, soluble cytokines, and immune cells (135). Host immune responses play a complex dichotomous role in lung cancer evolution. Although chronic inflammation has been shown to promote lung tumorigenesis (136, 137), cell-mediated immune responses hold the potential to eradicate premalignant and malignant cells (138). Recent studies indicate the presence of immune cells in premalignant and neoplastic lung lesions, suggesting that early-stage lesions elicit immune responses and subsequently develop means to circumvent them (15, 42, 47, 104, 109). It remains unclear why certain PMLs escape host immune surveillance over time and progress to invasive cancer whereas others regress.

Chronic dysregulated inflammation within the lung microenvironment may contribute to tumorigenesis through several mechanisms (136, 139, 140). For instance, cigarette smoke can trigger chronic pulmonary inflammation associated with aberrant expression of growth factors and cytokines, such as TGF-β, IL-1, IL-4, IL-6, IL-8, IL-10, IL-17, and IL-22. These, in turn, activate multiple inflammatory pathways, such as NF-κB (nuclear factor-κB) and STAT3 (signal transducer and activator of transcription 3), to promote tumorigenesis (136, 139, 140). Moreover, smoke injury leads to the accumulation of immune cells in the airways and peripheral airspaces before the development of preneoplasia. Such inflammation promotes a wound-healing microenvironment fueled by alternatively activated macrophages and immune suppressive monocytes and neutrophils (i.e., myeloid-derived suppressor cells) (140, 141). It remains unclear whether this classic myeloid inflammatory response is operative in peripheral lung preneoplasia in the absence of cigarette smoke. In established lung cancers, neutrophil infiltration has been correlated with reduced lymphocyte count and poor clinical outcomes (142–144). This inverse relationship between neutrophils and lymphocytes in lung cancers is statistically strongest in larger tumors. In contrast, there is evidence that tumor-associated neutrophils may promote lymphocyte proliferation in smaller tumors (e.g., 1 cm) (145).

AAH and AIS lesions typically possess a truncal driver mutation, most commonly in KRAS or EGFR (146, 147). As premalignant and malignant cells accumulate additional genetic mutations and post-translational aberrations, they become more immunogenic (148, 149). However, it is unclear when the immune system can recognize these lesions as foreign. Although KRAS mutation has been demonstrated to elicit antigen-specific immune responses in colon and pancreatic cancer (150, 151), it is uncertain whether common truncal driver oncogenes in lung preneoplasia elicit similar immune responses. Notably, oncogenic mutation frequency negatively correlates with major histocompatibility complex I (MHC-I) presentation in lung cancer (152). Therefore, recurrent oncogenic mutations, such as EGFR, tend to be biased toward poorly presented peptides.

Given that studying immune responses in lung preneoplasia is a relatively new field, several unanswered questions remain. We address these questions in the context of the role of the airway epithelium in sculpting the immune response and the role of immune surveillance afforded by antigen-presenting cells (APCs) and lymphocyte populations (Figure 2).

Activation of innate immune-driven inflammatory responses in the airway epithelium. Dysregulated activation of innate immunity in the airway mucosal epithelium, the first line of host defense against constant environmental and pathogenic insults (153), elicits chronic inflammatory responses that contribute to the initiation and progression of NSCLC (154). Indeed, chronic airway inflammation is a feature of patients with COPD who are at an increased risk (three- to ninefold) of developing NSCLC (155). Chronic airway inflammation associated with NSCLC is triggered primarily by prolonged tobacco smoke exposure, while other causal factors include bacterial infections and nonmicrobial agents such as particulate air pollution (86, 137, 156–159). However, significant knowledge gaps exist in our understanding of the molecular mechanisms by which inflammatory stimuli interact with the airway epithelium to trigger tumor-promoting immune responses.

Innate immune responses to microbial and nonmicrobial stimuli depend on a series of cell surface, endosomal, and cytosolic pattern recognition receptors (PRRs) that are expressed in immune and nonimmune (e.g., airway epithelial) cells (160, 161). PRRs are classified into several functionally and structurally conserved subfamilies, including TLRs (Toll-like receptors), ALRs (absent in melanoma 2–like receptors), and NLRs (NOD-like receptors) (162–164). Surprisingly, investigations into the role of PRRs in lung cancer are still in their infancy, with limited clinical data, some of which is contradictory (e.g., for TLR2), suggesting that tumoral expression of numerous TLRs

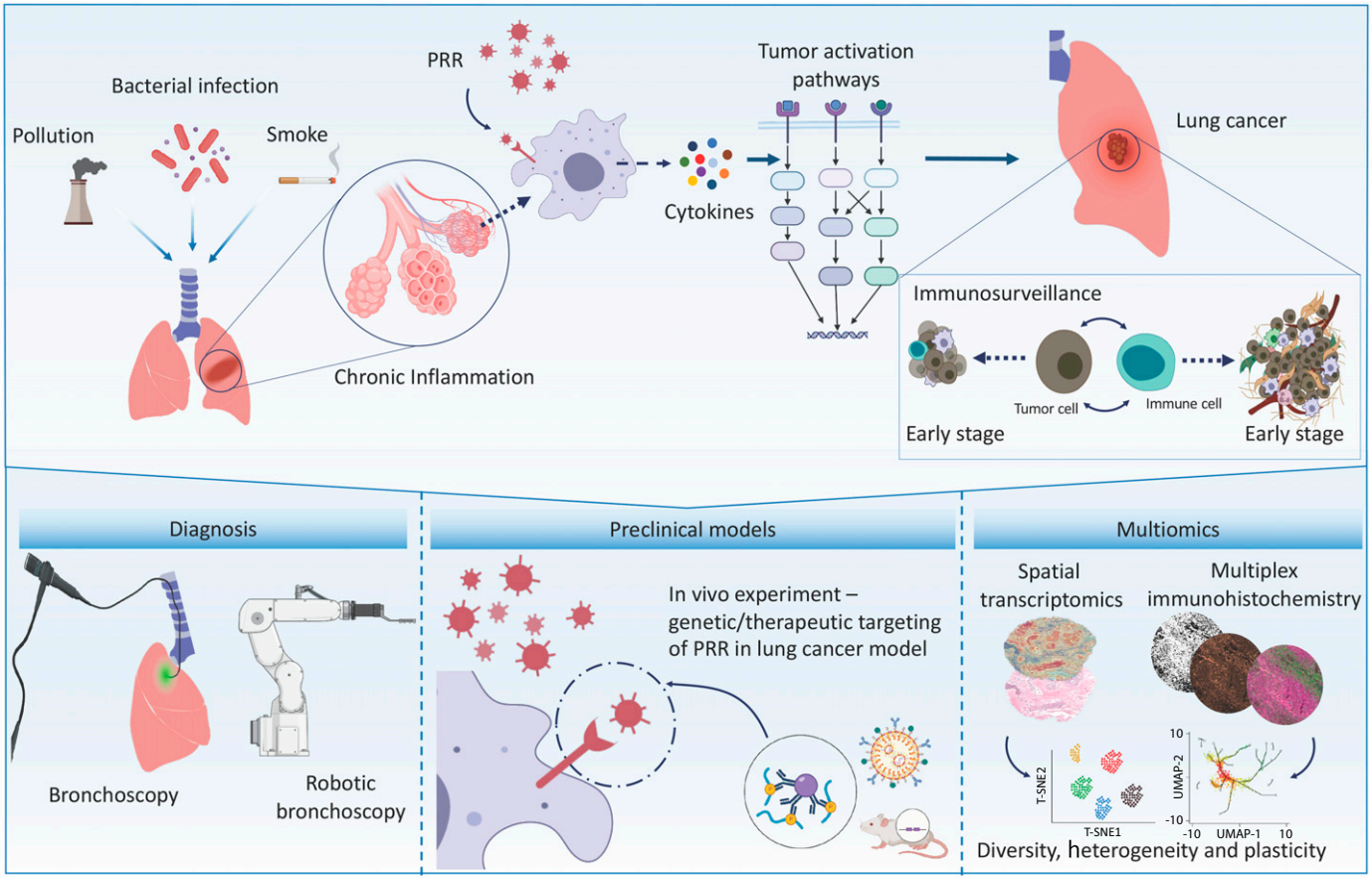

Figure 2. Roles of host precancer immune/stromal interaction in facilitating tumor development and progression. Dysregulated chronic inflammation, caused by pollution, infection, and smoke, induces aberrant expression of cytokines and accumulation of immune cells in the airways. This interplay of the immune system in chronic inflammation precedes neoplastic development and promotes lung cancer through the activation of multiple tumorigenic pathways. In addition, the interaction between immune cells and the surrounding tumor microenvironment influences host immune surveillance from early-stage tumors to invasive stages. Advanced bronchoscopy is used as a powerful tool for diagnosing and tracing the evolutionary trajectory of antitumor adaptive immune responses during lung tumor development. It also aids in exploring potential high-risk premalignant lesions, which may increase tumor invasiveness. Pattern recognition receptors (PRRs), crucial in the innate immune system for recognizing primary pathogens in inflammation, are poorly defined in the context of premalignant lung lesions. Therapeutic targeting of PRRs in preclinical lung cancer models is essential to uncover host–microbial interactions in this context. Using spatial omics approaches (spatial transcriptomics and multiplex immunohistochemistry) facilitates the definition of heterogeneity and cellular plasticity in the clinical trajectory and immunopathogenesis of premalignant lung lesions.

is upregulated in NSCLC patients and correlates with clinicopathological parameters, including tumor stage, improved or impaired survival outcomes, and chemoresistance (165–170).

The lack of clarity regarding PRRs in lung cancer is exacerbated by the paucity of *in vivo* studies using genetic and/or therapeutic targeting of PRRs in preclinical models. Some TLRs (TLR2, 4, 7, and 9) and their downstream signaling pathways (MyD88, IKK/NF-κB) were reported to drive tumor-promoting activities in the airway epithelium directly via intrinsic effects on cell proliferation and survival and indirectly via the recruitment and activation of infiltrating immune cells (e.g., myeloid-derived suppressor cells) to provide a chronically inflamed and invariably immunosuppressive TME (159, 166, 167). The requirement for these TLRs also extends to tumor-promoting inflammatory responses in airway epithelial cells induced by NSCLC risk factors, namely, cigarette smoke (containing the TLR4 ligand LPS) and nontypeable *Haemophilus influenzae* infection (159, 171–173). However, the complexities by which TLRs modulate lung tumorigenesis are highlighted by contrasting findings in the oncogenic $Kras^{G12D}$ NSCLC model, whereby TLR2 deficiency can either exacerbate or suppress lung tumor growth, reasons for which remain unclear (159, 169).

The magnitude and duration of host innate immune responses in the airway epithelium to microbial and environmental inflammatory stimuli promise to be a critical determinant in shaping the course of lung cancer. The recent discovery of a diverse lung microbiome whose dysbiosis is linked to lung carcinogenesis also suggests that microbial-sensing PRRs are potential critical conduits of host–microbial interactions in NSCLC (174). Although PRRs are attractive targets for drug development and biomarker discovery in lung cancer, significant knowledge gaps hamper our fundamental understanding of how airway epithelial PRRs contribute to carcinogenesis. From a clinical perspective, there is a clear need for more unified and comprehensive analyses of correlations between the expression and mutation profile of PRRs with clinical outcomes (e.g., therapeutic response and survival) across multiple lung cancer patient datasets incorporating known risk factors. The acquisition of robust clinical data will also complement and inform preclinical studies involving lung cancer mouse models with genetic and/or therapeutic targeting of PRRs. In this regard, future studies need to consider preclinical models that provide sufficient coverage of various molecular subtypes of NSCLC (e.g., *KRAS* mutant, *EGFR* mutant) coupled with exposure to risk factors (smoking, infection) (discussed further in objective 3). There is also a need to diversify the current scope of PRRs in lung cancer beyond TLR family members and include additional PRR subfamilies (e.g., NLRs, ALRs) implicated in other inflammation-associated epithelial cancers (161).

Key gaps and research questions in this area include the following: *II-1*) Is the myeloid enriched inflammatory response operative in smokers and nonsmokers alike? *II-2*) Is the heterogeneity of the myeloid compartment in PML lesions similar to that in advance disease? *II-3*) What is the functional impact of various myeloid compartments in the TME at the earliest stages of lung premalignancy? and *II-4*) What is the impact of chronic lung conditions such as COPD and interstitial lung disease on the TLR repertoire and the myeloid compartment of the PML microenvironment?

Cell-mediated immune surveillance. Host immune effectors impose continuous selective pressure on tumor cells throughout the evolution of lung cancer. The central tenant of this immunosurveillance is cancer cell recognition by antigen-specific T cells (138, 147). Genomic mutation in cancer cells may lead to the accumulation of abnormal proteins and the expression of neoantigens on MHC molecules on the cell surface. The presentation of these tumor antigens by professional APCs, such as dendritic cells (DCs), in conjunction with positive costimulatory signals, can activate host T cells in tumor-draining lymph nodes. Consequently, activated effector T cells circulate systemically and may eradicate cancer cells expressing MHC-bound tumor antigens, consistent with immunoediting. Tumor cell death can result in the release of additional tumor antigens in the TME, leading to further amplification and broadening of antitumor T-cell responses. This self-sustaining iterative process, called the cancer-immunity cycle, is crucial for successful antitumor immunity (175).

The pivotal role of DCs and antitumor T-cell responses in determining the clinical trajectory of lung cancer is highlighted by studies that have identified the presence of mature DCs, tertiary lymphoid structures with T follicular helper–like cells, and $CD8^+$ cytolytic T cells (CTLs) within the TME as positive prognostic indicators (176–179). Favorable responses to ICB have been associated with increased tumor mutational burden, presumably resulting in increased tumor antigens and elevated baseline $CD8^+$ CTL tumor infiltration (180–182). Increased IFNγ and CXCL9 signatures in the TME and a cellular module consisting of activated T cells and APCs have also been associated with improved clinical benefit of ICB (103, 183–187). However, our knowledge of the mutational, neoantigen, gene expression, and immune profiles that predict progression of pulmonary premalignancy at the earliest stages remains incomplete. This stems from the scarcity of longitudinally collected premalignant biospecimens and a paucity of murine models that adequately simulate the mutational and immune complexity of lung tumorigenesis.

The immunopathogenesis of early-spectrum lung cancer is anticipated to demonstrate significant interpatient heterogeneity, attributed to variations in patients' smoking and exposure histories, underlying lung disease, genomic drivers, mutational burden, and systemic immune status. In addition, there is insufficient understanding of the variability of host immune responses within synchronous lesions. Advances in bronchoscopy have empowered researchers to explore the evolutionary trajectory of antitumor adaptive immune responses during early lung tumorigenesis. Longitudinal studies evaluating squamous PMLs in the central airways, obtained via autofluorescence bronchoscopy, identified decreased gene expression in IFN signaling, antigen processing and presentation, and T cell–mediated immunity among progressive lesions compared with regressive lesions (80). These findings suggest the potential for identifying high-risk PMLs that may progress to LUSC. Increased T-cell exhaustion accompanied by a higher mutational and putative neoantigen burden was also observed in progressive carcinoma *in situ* lesions, suggesting that escape from immunosurveillance could be mediated by early upregulation of checkpoint inhibitors (13, 15, 80).

In contrast to LUSC, longitudinal studies in LUAD are lacking because of the peripheral distribution of these lesions. A study assessing FFPE early-stage LUAD and surrounding PMLs revealed increased

percentages of neoantigens shared between premalignant AAH lesions and the associated LUAD correlated with higher degrees of CD8 T-cell infiltration in AAH, suggesting potential immune recognition of neoantigens at the earliest points of LUAD development (42). These data must be interpreted cautiously because of the presumption that spatially distinct AAH lesions are representative of PMLs preceding transformation to invasive cancer.

Future studies are warranted to better define the complex evolution of tumor antigen profiles and host immune responses in early-spectrum lung cancer. Research efforts should focus on elucidating the functional significance of host T cells in lung premalignancy. Technological advances in robotic bronchoscopy are poised to enhance accessibility to early-spectrum LUAD. These efforts will be facilitated by the Human Tumor Atlas Network, a National Cancer Institute Cancer Moonshot initiative, which includes the Lung Pre-Cancer Atlas Project. The network is constructing three-dimensional atlases of the dynamic cellular, morphologic, and molecular features of lung cancers as they evolve from precancerous lesions to advanced diseases (188–190). To attain a more nuanced understanding of cell–cell interactions and immune niche within the TME of lung premalignancy, the incorporation of spatial transcriptomics is required. Spatial analyses will also yield insights into the impact of intratumoral heterogeneity in cellular and antigen profiles on host immunosurveillance. Leveraging artificial intelligence in conjugation with omic approaches holds promise for expediting the identification of immunophenotypes that denote lesions at the highest progression risk.

Key gaps and research questions in this area include the following: *II-5*) Do immunologic features differ between PMLs that regress and those that progress to invasive cancer? *II-6*) What are the immune determinants of progression in pulmonary premalignancy? *II-7*) Are the mechanisms underlying immune escape similar in LUAD and LUSC? *II-8*) How does tumor mutational burden affect the trajectory of adaptive immune responses in premalignancy?

The heterogeneity of lung premalignancy highlights the importance of precision medicine in the development of immune-based cancer interception strategies. This process will require two key components: *1*) identifying lung PMLs with the highest risk for transformation to invasive cancer and *2*) elucidating the specific features of immune suppression operable in each lesion.

Studies of early-spectrum LUAD have revealed T-cell infiltration into lung premalignancy, accompanied by upregulation of immune checkpoints (13, 42). This observation suggests that ICB could be a promising strategy for cancer interception at its early stages. However, the full spectrum of mechanisms involved in immune evasion in premalignancy remains unknown.

Several critical questions remain unanswered: *II-9*) What immune phenotypes prompt interventions, and what immune-regulatory interception strategies could be applied? *II-10*) Are "immune-desert" or "immune-excluded" phenotypes (191), known to confer resistance to ICB in NSCLC, operative in high-risk pulmonary lesions? *II-11*) Does the paucity of type 1 conventional DC infiltration in early lung cancer (48, 109, 192) imply inadequate antigen presentation and T-cell activation against tumor antigens? and *II-12*) Does exploring *in situ* vaccination with autologous DCs, previously shown to induce systemic tumor-specific T-cell responses in patients with NSCLC (193), hold potential for individuals identified with high-risk PMLs exhibiting impaired antigen presentation?

Recent studies have unveiled critical genomic alterations in NSCLC contributing to immune evasion. Frequent homozygous deletions of chromosome 9p21.3, which includes the *CDKN2A* and *B* tumor suppressors and a cluster of 16 IFN-I genes, have been identified in NSCLC (194). This genetic alteration correlates with decreased expression of CXCL9, a reduction in tumor-infiltrating $CD8^+$ CTL receptor diversity, and increased resistance to ICB (195). In addition, *LKB1*-inactivating mutations, which lead to the downregulation of stimulator of IFN genes (*STING*) and CXCR2-mediated recruitment of suppressive neutrophils in the TME, have been identified as primary drivers of resistance to ICB in *KRAS*-mutant NSCLC (196–198). The understanding of the lung premalignancy genomic landscape and its correlation with the immune contexture of the TME remains limited. Future studies in this area will provide insights, facilitating the identification of patients for personalized therapies that enhance adaptive immune responses by mitigating tumor-mediated immunosuppression.

Related research questions to this objective include the following: *II-13*) What are the roles of different cell clusters, immune or stromal, in preneoplastic lesions with different driver or codriver mutations? *II-14*) What are the genomic drivers of immune escape in PMLs? and *II-15*) What are the roles of different cell clusters, immune or stromal, in preneoplastic lesions with different driver or codriver mutations?

Objective 3: Proposing New Strategies and Opportunities to Intercept the Progression of Premalignant Disease

Themes in objectives 1 and 2 of this research statement explore mechanisms of preneoplastic progression that could be applied for interception. In objective 3, we focus on strategies to model and block cancer development by further exploring the PML histopathology and diagnosis; risk assessment, including biomarkers of disease development and PML progression; and the evolution of chemoprevention trials.

PMLs sampling and profiling. Given the rarity of premalignant human lung samples and the need for long-term follow-up of patients, prioritization, funding of biobanks, and large-scale efforts to molecularly profile specimens with a single-cell and spatial resolution are critical. As stated previously, most aPML studies examine tissues acquired during lung cancer resection (42–44, 46, 146, 199–207). Currently, longitudinal sampling of LUSC PMLs is possible, and in the future, electromagnetic navigation and robotic bronchoscopy should improve access to LUAD lesions. As previously mentioned, the PreCancer Atlas has facilitated an organized approach to the collection, categorization, and characterization of PMLs (188–190). The ability to sample lesions longitudinally in the absence of lung cancer will allow the identification of molecular changes that occur over time as the lesions resolve or progress. However, lesions can have a long latency period, so repeated sampling may miss important transitional stages. In addition, biopsy involves partial or complete resection of the lesion, and recurrence of the lesion at the biopsy site may occur at different times, making histologic outcome measures challenging. Using histologic changes as a surrogate for disease progression is also complicated by lesion

heterogeneity and variability among pathologists in grading. As research advances, there is an opportunity to redefine the histologic spectrum, and thus disease progression using machine learning models derived from a variety of data such as molecular alterations, digitized pathology images, and clinical data to obtain a more holistic view of the state of the lesions and their microenvironment.

In most studies profiling human PMLs published to date, the lung etiologic field of injury is not simultaneously sampled and profiled. The lung injury field is the sum of molecular and cellular changes associated with the host response to injury caused by carcinogen exposure (35, 208–219). Studies examining DNA have described somatic changes, copy-number aberrations, genomic signatures, and telomere shortening in the lung region that are tumor independent (40, 86, 220, 221). How the lung injury field influences the ability of lesion development and progression, or how dynamic the lung injury field is in the presence of lesions, has implications for the development of noninvasive biomarkers of prognosis and response to treatment obtained from nasal brushings, sputum, or blood biospecimens and CT and pathology images that are all influenced by lung field changes. There is an opportunity for biobanking programs to collect noninvasive biospecimens and imaging data in addition to lesion biopsies so that important lesion-specific biology can readily be translated to less invasive tissues.

The key question is, *III-1*) Can less invasive biospecimens predict the presence of premalignant disease and be used to follow the response to preventive interventions?

Biomarkers of risk assessment, premalignancy, and disease progression. Clinical assessment of lung premalignancy and progression continues to evolve. Recognized clinically evident PMLs include AAH and AIS for LUAD and squamous metaplasia, dysplasia, and carcinoma *in situ* for LUSC (11, 222). The ideal biomarker of premalignancy would help clinicians determine *1*) if a lesion was benign, premalignant, or fully malignant; *2*) if a PML could aggressively progress to invasive malignancy; and *3*) if the PML would respond to targeted therapy. The pathway to developing biomarkers for lung cancer has been clearly laid out in prior ATS research statements (223), and the same stages of biomarker development should be followed for biomarkers of premalignancy. In addition, the 2017 ATS research statement delineated three categories of biomarkers: *1*) risk prediction, to determine which individuals are at higher risk for developing lung cancer so that they can be directed to screening; *2*) cancer detection, for use within the screening or symptom evaluation setting; and *3*) diagnosis, to be used in the evaluation of a lung nodule, mass, or other imaging finding. A biomarker of risk prediction or detection for PMLs would have the same population and use as for lung cancer and would therefore fall into the appropriate category from the 2017 statement. However, a diagnostic or prognostic biomarker of premalignancy would be useful to guide the form and/or aggressiveness of care. Currently, all U.S. Food and Drug Administration–approved biomarkers for lung cancer early detection and/or diagnosis are developed for estimating the risk of developing lung cancer or for risk stratification of pulmonary nodules.

Imaging biomarkers. Chest imaging, regardless of whether performed in a screening setting or not, is still the best clinical tool for detection of PMLs, with upward of 30% of scans revealing pulmonary nodules (224, 225). Many PMLs are large enough to be clearly seen on CT imaging studies; for example, AIS is commonly between 5 and 20 mm in largest diameter, which would be captured with standard low-dose CT screening slick thicknesses of 2.5 mm or less. LUAD-spectrum lesions often show a characteristic appearance on chest CT scans, where they may appear as persistent ground-glass opacities (GGOs) or part-solid lesions, but they may represent focal fibrosis or infection (222, 226). There is significant debate on monitoring and management of these abnormalities, with increasing recognition that many PMLs may never progress and thus contribute to overdiagnosis and overtreatment (227, 228). They may exhibit indolent behavior (including slow growth), with doubling times often far greater than one year (222, 229). However, these lesions also represent only a subset of LUAD premalignancies. On the basis of clinicopathological studies of resected GGOs, these lesions tend to have excellent prognosis (230, 231). In contrast, cancers detected by lung cancer screening after initially negative results on CT exhibit poorer survival than those detected after initially positive results on CT (232). This suggests that many aggressive LUADs originate from PMLs that either are not evident radiologically or have short dwell times as pure GGOs. The histology of squamous-spectrum lesions is not evident on CT imaging but can be detected during bronchoscopy, with techniques such as autofluorescence and narrow-band imaging bronchoscopy increasing diagnostic yield (233).

Because of the unique metabolic nature of PMLs, as discussed in objective 1, PET could play a role in the clinical evaluation of potentially PMLs. However, FDG PET has limited sensitivity and specificity for the early detection of lung cancer currently. Several reports describe the lack of PET–CT sensitivity in GGOs, which may limit its performance in LUAD PMLs (234, 235). As many pure ground-glass lesions are inflammatory, modest FDG uptake observed in premalignancy could be confounded, with some studies even reporting a negative correlation between FDG uptake and malignancy risk (235, 236). In addition, PET has limited specificity in geographic regions with endemic fungal disease.

An area showing enormous promise is radiomic analyses of chest imaging, both standard CT and PET–CT. In standard CT, multiple algorithms can discriminate benign from malignant nodules and predict lung cancer, even from areas lacking apparent radiographic abnormalities (237, 238). Radiomic algorithms in both CT and PET–CT, often based on artificial intelligence selection of quantitated CT imaging features, help automate and standardize measurement of lung nodule dimensions, texture, vascularity, and borders. Radiomic assessment of LUADs across the spectrum has shown the ability to discriminate among MIA, AIS, and fully invasive LUAD and to predict the aggressiveness of such lesions (239). These approaches standardize the measurement of doubling time and identify high-risk features, which may facilitate the discrimination of indolent lesions beginning to progress. However, numerous studies report discovery of high-risk features in GGOs, but to date, no prospective validated approaches have shown the ability to predict malignant transformation (240). As with biomarkers, there is a lack of trials evaluating performance of radiomic approaches to assist in predicting which GGOs will progress to invasive cancer. Integration with other imaging modalities (e.g., high-resolution CT) or integration of PET–CT findings into machine learning

algorithms may be necessary to counteract these observations (241).

The knowledge gaps and main research questions are the following: *III-2*) Can imaging biomarkers and radiomic analyses correctly differentiate PMLs from other lung lesions? and *III-3*) Can these approaches help identify PMLs that will evolve into clinically relevant diseases?

Molecular biomarkers. Several studies have reported the utility of biomarkers for refining lung cancer risk, including performance in samples taken 12 months or more before lung cancer diagnosis (242–244). Several biomarkers are approved by the Food and Drug Administration for the discrimination of indeterminate pulmonary nodules (245), including tests that measure circulating proteins, autoantibodies, and an endobronchial genomic classifier. To date, these tests have shown limited sensitivity for early-stage disease, possibly hindering their ability to discriminate premalignant progression (246, 247). However, no studies have directly correlated biomarker performance to defined premalignancy, and this is an opportunity area for additional studies. In bronchial metaplastic lesions, gene expression profiling showed alterations associated with progression (10) and distinct molecular subtypes (80). Several biomarkers have been evaluated for performance in GGOs but lack validation in independent cohorts (248, 249).

Biomarkers that evaluate the immune response or the field of cancerization may be well suited to characterize PMLs. For example, in bronchial metaplastic lesions, gene expression profiling showed alterations associated with progression (10) and distinct molecular subtypes (80). Cancer-associated gene expression changes in the bronchial airway field have been used to develop a biomarker to aid in the clinical diagnosis of lung cancer (250). This concept was recently extended to the nasal epithelium to diagnose lung cancer in patients with indeterminate pulmonary nodules (251). These results confirm a common field of injury, but translating bronchial to nasal gene expression remains exploratory, and considerable work is needed to better define the molecular and cellular relationships throughout the field. Nasal gene expression is one biomarker that may eventually prove useful in lung cancer interception trials.

Although noninvasive gene expression profiles may serve as a biomarker for lung cancer detection, they do not yet correlate with the intricate immune biology of premalignancy. To bridge this gap, longitudinal studies should integrate liquid biopsies and possibly nasal gene expression with local immune phenotypes of premalignancy alongside clinical outcomes. These studies may facilitate the development of noninvasive biomarkers capable of identifying the specific nature of immunosuppression in high-risk lesions.

The related key knowledge gaps and emerging questions are as follows: *III-4*) Can blood and tissue biomarkers refine the risk of developing lung cancer in prospective trials? and *III-5*) Do blood biomarkers reflect the internal biology of PMLs enough to guide early intervention, including chemoprevention and immunointervention?

Biomarker development cohorts. Pathologically confirmed precancerous lesions are much rarer than benign or fully malignant lesions, which means that these are underrepresented in both imaging and biobanked cohorts. The National Cancer Institute–funded Lung Pre-Cancer Atlas Project is one step toward developing research cohorts to answer these questions, but more curation of pathologically confirmed PML cohorts will be necessary for premalignant biomarker development.

Moving forward, lung cancer screening and nodule cohorts present an opportunity to evaluate biomarker performance through disease progression. For individuals undergoing longitudinal imaging, within-subjects analyses could be a potent tool to better evaluate disease trajectory, response, and recurrence. As molecular profiling becomes more prevalent, biomarkers can be associated with mutational and immune microenvironmental variation. For instance, as a large percentage of GGOs contain *EGFR*-activating mutations, biomarkers that reflect tumor mutational background (including circulating DNA) may help direct treatment in early disease (252). Longitudinal biomarker trends may also help assess treatment response and recurrence. An intriguing area for future exploration will be whether predictive biomarkers can identify those who may benefit from a chemopreventive regimen.

Critically, a clinically useful biomarker of premalignancy would provide information about the lesion to inform care differently than a diagnostic biomarker. To guide the development of PML biomarkers, several key clinical questions must be defined. For example, should a biomarker of premalignancy be developed, would it provide actionable data different from that provided by biomarkers of cancer probability? For example, an indeterminate pulmonary nodule that is highly suspicious for cancer should undergo a biopsy to confirm malignancy. If a biomarker, whether blood or image based, were to classify a lesion as "premalignant" with high accuracy, the next steps in managing the patient would still likely include a tissue biopsy to guide treatment, followed by an intervention or close monitoring. To efficiently guide the development of premalignant biomarkers, clinical use scenarios should be developed. However, in the future, should therapies specific to PMLs be developed, a premalignant biomarker may accurately guide the use of these therapies, which could avoid overtreatment of PMLs.

Hence, the key knowledge gaps and research questions include the following: *III-6*) Can biomarkers (blood based, radiomic) determine the presence, grade, and progression of premalignant airway lesions? *III-7*) Can biomarkers be developed to determine response to medical prevention agents, and what are the best clinical use scenarios? and *III-8*) Can noninvasive biomarkers predict the nature of immunosuppression in early-spectrum lung cancer?

Opportunities for preclinical premalignant tools development. Mouse models of LUAD development mostly rely on oncogene induction (e.g., KRAS), tumor suppressor loss or mutation (e.g., p53, lkb1, EGFR), or the combination of both oncogene activation and tumor suppressor loss (e.g., KRAS–p53 [KP] mouse model) as well as carcinogen exposure (e.g., nicotine-derived nitrosamine ketone, benzopyrene, urethane). An important study by Westcott and colleagues demonstrated that the oncogene activation and tumor suppressor loss–driven and carcinogen exposure–driven mouse models of LUAD have very distinct landscapes of mutations and copy number changes (253). For instance, KRAS-driven lung tumors in animals had markedly lower somatic mutation burden compared with carcinogen-driven LUADs but higher degrees of aneuploidy (253). To recapitulate the mode of LUAD pathogenesis observed in humans, mouse models of LUAD should ideally comprise recognizable and progressively advancing pathological stages that include aPMLs and their malignant conversion to

locally invasive LUADs. The KP model shows a progressive spectrum of noninvasive early lesions (hyperplasias and adenomas) to locally invasive LUAD (57). Yet the early lesions do not fully capture the spectrum of aPMLs described during human LUAD development. Also, in humans, PMLs show varying degrees of progression (time and extent) to LUAD, whereas early lesions in the KP model are short lived. Alternatively, mouse models with carcinogen exposure (254) enable the analysis of the full spectrum of PMLs before LUAD formation. The downside of these models is that they do not capture the mode of LUAD pathogenesis found in nonsmokers (such as those with EGFR mutations). Additional preclinical models need to be developed and refined.

Understanding the steps that govern the transition of aPMLs to invasive LUAD can be advanced with a better understanding of TME interactions. Studies should strive to deconvolute the paracrine and juxtracrine signaling pathways that affect immunosuppression, inflammation, angiogenesis, and fibroblast activation, as described in objective 2. For example, a recent study used spatial transcriptomics analysis (255) to examine the microenvironment in KP-mutant lung cancers perturbed with deletion of TGF-R2 among 35 genes. Loss of TGF-R2 in tumors resulted in invasive remodeling with stromal fibroblast enrichment. These observations demonstrate key biological properties that characterize invasiveness acquisition in LUAD, the role of altered TGF-β signaling in driving this process, and the advantages of technical advances in genomics that permit a high-resolution approach to examining genomic and immune alterations that mitigate confounding by intratumoral heterogeneity.

Identifying the key knowledge gaps also highlights the need for the simultaneous development of preclinical models of lung carcinogenesis to study progression from premalignant to early lung cancer. Models should expand beyond common oncogene-driven models to include clinically relevant exposure models (e.g., carcinogens, e-cigarettes, and $PM_{2.5}$ exposures). Given the impact of inflammation and immunity on carcinogenesis, these should, preferably, be immune-competent models. In addition, given the mechanistic and correlative causes of NSCLC in concurrent lung and immune-deficient diseases known to increase lung cancer risk, such as COPD, interstitial lung disease, post-transplantation immunosuppressed and long-term HIV infection, preclinical models that can be used to study lung cancer development in the context of these diseases are critically needed. Finally, research should focus on the mechanistic impact of aging-related processes on DNA damage response and lung carcinogenesis. Mechanistic evaluations should include the evaluation of progenitor cell function and environment–immune interactions in carcinogenesis. Appropriately designed single-cell and spatial transcriptomic and proteomic characterizations of these preclinical models that go beyond descriptive measures will be potentially powerful tools to study early clonal progression and the mechanistic downstream impact of specific cell types and their interactions and to identify similarities and differences with human lung PMLs and tumors.

Novel cell culture systems that can be easily manipulated are also needed to help identify mechanisms of disease initiation and progression observed in preclinical models (232). For example, the inclusion of TME in three-dimensional cell culture may better recapitulate disease. Lung cancer organoids (LCO), grown on Matrigel (Corning), have been shown to model patient tumor heterogeneity, recapitulate primary lung tumor structure, and maintain original genomic alterations and therapeutic response (256). In addition, LCOs may be useful to study tumorigenesis, metastasis (257), and cell–cell interactions in the TME; allow quick genetic alteration–based studies (e.g., clustered regularly interspaced short palindromic repeats) (258); and facilitate high-throughput drug screening that may predict therapeutic response in patients (259). However, very few studies have used LCOs to investigate early oncogenic events during cancer initiation. High-throughput time-course studies in the complex coculture systems of LCOs hold immense potential for identifying the underlying genetic and epigenetic changes that govern cell–cell interaction networks over time and better capture how a concerted effort of the tumor cells and TME cells promote tumor initiation and provide means to sustain tumor growth (discussed extensively in objective 2).

Key research questions that must be answered in this area include the following: *III-9*) How can we best recreate LUSC and LUAD PMLs in murine models and determine features (both intrinsic and immune related) that predict progression to cancer? and *III-10*) How can we best model the spatial and temporal heterogeneity of the lesions preclinically and clinically?

Chemoprevention: prior, current, and future studies. Past and present clinical trials. Historically, phase III clinical trials attempting the prevention of initial lung cancer in high-risk smokers (summarized in Table 2) have not been positive (260–263). Instead, trials of β-carotene showed increased lung cancer risk and mortality (264), while a trial of 13-*cis* retinoic acid showed possible increased recurrence and mortality in current smokers (265). Additional data with other vitamins, aspirin, and micronutrients, all negative, were summarized in a recent Cochrane review (266).

As a result of past experience, subsequent clinical efforts have turned to phase II trials intended to demonstrate safety and preliminary efficacy. These trials have the potential to identify efficacious strategies more rapidly by focusing on intermediate endpoints (rather than cancer incidence) and by enrolling a smaller number of high-risk participants (e.g., 50–150) in trials of shorter duration (e.g., 6 mo). The prototype of these trials is the study of inhaled budesonide in individuals with bronchial dysplasia (267). On the basis of abundant data implicating inflammation in lung carcinogenesis, animal carcinogenesis models, and epidemiologic data, this trial monitored both the central airways via bronchoscopy and the peripheral lung via CT screening (268). Although the 6-month intervention did not result in dysplasia regression, there was increased regression of CT-detected nodules (a secondary endpoint). This led to new clinical trials focusing on individuals with indeterminate pulmonary nodules undergoing low-dose CT for screening. The first trial used inhaled budesonide for one year in individuals with solid or subsolid indeterminate nodules. Although the findings were initially negative, long-term follow-up showed a statistically significant, albeit small, persistent decrease in the size of ground-glass, but not solid, nodules (269, 270). Although the relevance of this finding to the subsequent development of cancer remains unclear, some GGOs are precursors to LUAD (271). A subsequent trial focused only on subsolid indeterminate nodules and used low-dose aspirin (272). Although its results were also negative, that trial helped establish the methodology for performing LUAD prevention trials.

Table 3 summarizes ongoing and recently completed early-phase trials that

Table 2. Phase III Lung Cancer Chemoprevention Trials

Trial Name (Reference)	Cohort	Sample Size	Intervention	Primary Endpoint	Effect
ATBC (260)	Male smokers, ages 50–69 yr	29,133	AT 50 mg/d vs. BC 20 mg/d vs. both vs. neither × 5–8 yr	Primary lung cancer: BC ↑ 18%, AT ↓ 2%	Harmful
CARET (261)	Smokers (current and former) and asbestos workers	18,314	BC 30 mg/d + retinol 25,000 IU/d vs. placebo × 5 yr	Primary lung cancer: RR, 1.28 Lung cancer mortality: RR, 1.46	Harmful
Isotretinoin Intergroup Trial (262)	Resected stage I NSCLC	1,166	Isotretinoin 30 mg/d vs. placebo × 3 yr	SPT: HR, 1.08 Mortality: HR, 1.07	Null
ECOG 5597 (263)	Resected stage I NSCLC	1,772	Se 200 μg/d vs. placebo × 48 mo	SPT Lung cancer: SPT = 1.62 vs. 1.30 per 100 person-years (Se/placebo) Overall: SPT = 3.54 vs. 3.39 per 100 person-years	Null
Physicians' Health Study (264)	Male physicians, ages 40–84 yr	22,071	BC 50 mg qod vs. ASA 325 vs. both vs. placebo × 12 yr	BC: lung cancer incidence Current smokers: RR, 0.90 Former smokers: RR, 1.0 Never-smokers: RR, 0.78	Null
EUROSCAN (265)	Resected NSCLC or H&N cancer	2,592	RP 300,000 IU/d × 1 yr, then 150,000 IU/d × 1 yr vs. NAC 600 mg/d × 2 yr vs. both vs. no intervention	5-yr OS: 71% vs. 72% (NAC vs. no NAC); 70% vs. 73% (RP vs. no RP); no effect on second primary cancer	Null

Definition of abbreviations: ASA = aspirin; AT = α-tocopherol; ATBC = Alpha-Tocopherol, Beta-Carotene Cancer Prevention; BC = β-carotene; CARET = Beta Carotene and Retinol Efficacy Trial; ECOG = Eastern Cooperative Oncology Group; EUROSCAN = European Study on Chemoprevention With Vitamin A and N-Acetylcysteine; H&N = head and neck; HR = hazard ratio; NAC = N-acetylcysteine; NSCLC = non–small cell lung cancer; OS = overall survival; qod = every other day; RP = retinyl palmitate; RR = relative risk; SPT = second primary tumor.

Table 3. Ongoing Early-Phase Lung Cancer Chemoprevention Trials

Agent	ClinicalTrials.gov Identifier	Cohort	Primary Endpoint
Canakinumab (Can-Prevent-Lung trial)	NCT 04789681	High-risk pulmonary nodules	Nodule regression
EGF-rP64K/montanide ISA 51 (CIMAvax-EGF) vaccine	NCT 04298606	High risk for lung cancer or lung cancer survivors	Antibody response, safety, molecular biomarkers
Black raspberry nectar (BE WELL)	NCT 04267874	Current and former smokers	Feasibility, effect on inflammatory biomarkers (nasal brushings), and stool microbiome
MUC1 peptide-poly-ICLC vaccine	NCT 03300817	Current and former smokers undergoing CT screening	Immunogenicity, safety
Pembrolizumab (IMPRINT-Lung Trial)	NCT 03634241	Indeterminate pulmonary nodules, with or without a history of lung cancer	Regression of high-risk nodules
Nivolumab	NCT 03347838	Current and former smokers with sputum atypia or histories of NSCLC or HNSCC	Improvement in endobronchial histology
Sulforaphane	NCT 03232138	Former smokers	Bronchial dysplasia, cell proliferation, apoptosis
Metformin	NCT 04931017	Overweight and obese former smokers	PD-1 expression on pulmonary Treg cells
Lovaza and curcumin C3 complex	NCT 03598309	Current and former smokers undergoing CT screening with lung nodules	Change in nodule size, rate of ⩾4-mm nodules

Definition of abbreviations: BE WELL = Black Raspberry Beverage Working to Prevent Lung Cancer; Can-Prevent-Lung = Canakinumab for the Prevention of Lung Cancer; CT = computed tomography; HNSCC = head and neck squamous cell carcinoma; IMPRINT-Lung = Single-Arm, Phase II of Immunotherapy with Pembrolizumab for the Prevention of Lung Cancer; NSCLC = non–small cell lung cancer; Treg = regulatory T.

still need to report results. This new generation of trials focuses more on immune therapies, including checkpoint inhibitors, and reflects the importance of immune dysregulation during early lung cancer development. Furthermore, the recognition of worse outcomes for current versus former smokers in prior phase III trials has resulted in an exclusive focus on former smokers in several studies. To further direct interventions to more homogeneous cohorts, some trials are using risk calculators, such as Modified Prostate, Lung, Colorectal, and Ovarian Cancer Risk-Prediction Model, which goes beyond smoking history to identify high-risk individuals. One ongoing trial is targeting high-risk (per the Modified Prostate, Lung, Colorectal, and Ovarian Lung-Cancer Risk-Prediction Model risk calculator) obese or overweight former smokers to optimize patients most likely to benefit from the intervention (NCT 04931017). Some trials also examine gene expression profiles from the abnormal field to develop predictive biomarkers to better deliver interventions to those most likely to benefit (i.e., precision prevention) (273). More precise delineation of risk, coupled with a better understanding of the molecular and immunologic underpinnings of carcinogenic progression, can bring precision medicine to lung cancer prevention.

True precision prevention approaches require considerably more knowledge than we currently have. Multiple challenges, both biological and trial related, have thus far limited precision prevention. Among the biggest gaps is our limited understanding of the early events in lung carcinogenesis. As mentioned above, a longitudinal study of premalignancy is difficult because these lesions are generally very small and are not routinely detected or excised. Nevertheless, it is critical to understand the molecular and immunologic dysregulation to identify intervention targets. A second major biological challenge is accounting for heterogeneity, both between and within individuals. Cancer evolution involves spatial and temporal diversity, with different processes predominating during different phases (e.g., tobacco-driven mutagenesis occurs early, whereas APOBEC-associated changes occur later) (274). Strategies targeting the entire carcinogenic process are needed. A third challenge is the need to understand the host response to optimize efficacy and limit toxicity.

There are also important trial-related challenges in drug development (275). Whereas phase III cancer endpoint trials are lengthy and require large numbers of participants, early-phase trials are challenging because they frequently require invasive procedures to obtain biopsies for endpoint assessment. They depend on surrogates for cancer incidence that are difficult to validate, providing efficacy information that still requires subsequent phase III trial confirmation. Although trials focus on high-risk cohorts (e.g., as defined by tobacco exposure, typically a minimum of 20–30 pack-years), the cohorts are often heterogeneous nevertheless, and thus interventions that are effective for subgroups may be missed. The more homogeneous the cohort, however, the greater the difficulty in accrual as the cohort pool size diminishes and the lesser the generalizability to the population at large. Finally, it is of paramount importance to consider the risk–benefit balance, since only a portion of the targeted at-risk population will ever develop lung cancer. Given the potential long-term use, interventions need to be safe and tolerable. Alternatively, regional drug delivery (e.g., by inhalation) or intermittent dosing schedules have the potential to optimize the risk–benefit balance.

Key research questions in the area of clinical trial that need to be answered include the following: *III-11*) Can we improve the understanding of early events in lung carcinogenesis (epithelial and immune dysregulation) to identify intervention targets? *III-12*) Can noninvasive biomarkers be developed that predict presence of PMLs, and can these be followed over time rather than performing longitudinal biopsies? and *III-13*) Can we develop medical interception strategies with good risk:benefit ratios and study agents in those with lesions at risk for progression?

Conclusions

As outlined in this statement, the progression from PMLs to advanced cancer represents a dynamic and multifaceted process influenced by a variety of carcinogenic stimuli. This progression is characterized by a complex interaction between epithelial and tumor cells and stromal cells mediated by protumorigenic factors and signaling pathways. Compounding the complexity of this process is the dual role of host immune responses, where chronic inflammation acts as a protumorigenic force, while cell-mediated antitumor immunosurveillance functions to eradicate neoplastic lesions. In this context, we have identified critical knowledge gaps and formulated specific research questions essential for advancing our comprehension of the mechanisms underlying PML progression. There is a compelling need for comprehensive exploration of several key areas to enhance our better understanding of PML progression, develop preclinical models, establish effective screening strategies, and develop early detection biomarkers. Advances in these areas are anticipated to significantly contribute to the development of preventive measures that may reduce lung cancer incidence. Furthermore, these efforts will facilitate the identification of new molecular targets amendable to early interventions thereby enabling precision cancer prevention trials among high-risk populations. Ultimately, the aim of these initiatives is to provide novel therapeutic modalities and prognostic biomarkers that improve patient responses and survival rates. ■

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