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# Advances in lung cancer basic and translational research in 2025 – Overview and perspectives focusing on non-small cell lung cancer

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## Abstract

Basic and translational research in lung cancer is a rapidly evolving field with transformational impact in early detection, diagnosis, therapeutic development and personalization of care. Recent advances have greatly increased our understanding in the molecular genomics, proteomics, pathogenesis and cellular biology of this deadly malignancy. The International Association for the Study of Lung Cancer (IASLC) recently formed a Basic and Translational Science (BaTS) Committee to further enhance the scientific leadership of IASLC in thoracic cancer

research. This review by members of the committee highlights the breadth of current research in NSCLC, with a focus on molecular risk factors and processes in tumorigenesis, heterogeneity, phenotypic plasticity, metabolic reprograming, immunobiology, the immune microenvironment and microbiome. This review also identifies future research areas that may lead to further improvement in survival outcomes and curative therapies especially for patients with advanced NSCLC.

### Keywords

NSCLC; translational research; genomics; early lung cancer; states of art; tumor heterogeneity; cancer plasticity; immune microenvironment; TME

## Introduction

Lung cancer is marked by complex genomic and epigenomic aberrations dysregulating the cellular signaling mechanisms and functions of tumor cells, and their interaction with the tumor microenvironment (TME). Non-small cell lung cancer (NSCLC) is commonly driven by a complex interplay of many biological pathways, including among others, signal transduction, DNA repair, chromatin modifications, metabolism, and immune checkpoint pathways. Breakthroughs that have occurred in basic and translational research during the last decades have not only enhanced our understanding of the biology and disease mechanisms in lung cancer, but they have also translated into the development of novel and personalized therapeutic strategies, which have improved the overall survival and quality of life for lung cancer patients. In this context, the Basic and Translational Science (BaTS) Committee of the International Association for the Study of Lung Cancer (IASLC) provides a review of the recent advances in our understanding of NSCLC at a cellular and molecular level, and its interactions with the tumor microenvironment (TME). We highlight the breadth of current lung cancer research and identify future directions towards advancing scientific innovation to improve patient outcomes and curative therapies.

## Inherited susceptibility to lung cancer

Major progress has been made to elucidate both single-gene and polygenic inheritance of lung cancer.[1,2] A significant proportion (4–15%) of patients with lung cancers harbor pathogenic germline variants in cancer genes.[3,4,5] These are low-frequency variants with high or moderate-penetrance genes, such as *BRCA1, BRCA2, CHEK2, ATM, BAP1, EGFR* and *TP53*. The genome-wide association studies (GWAS) have identified more than 60 lung cancer susceptibility loci.[1,6,7] The potential clinical applications of these GWAS results are the development of polygenic risk scores (PRSs), which aggregate information from multiple genetic variants to quantify an individual's genetic predisposition to lung cancer. Different PRSs were demonstrated as predictors of lung cancer independent of conventional clinical risk factors.[8,9] A genome-wide PRS, leveraging the full GWAS data to quantify genetic predisposition, was recently demonstrated to outperform previously reported PRSs.[10] Although the predictive power of the best-performing PRS does not surpass smoking history, it is comparable to other factors used in lung cancer risk models,

such as age and sex.[8,10] Moreover, the polygenic background has been shown to modulate the risk associated with smoking. However, it remains controversial whether the risk stratification benefits of PRSs are more meaningful in never or ever-smokers.[11,12,13,14] Nonetheless, advances in germline genetic testing and PRS provide new opportunities to identify individuals at high risk of lung cancer and we expect this emerging genetic knowledge to be increasingly integrated into lung cancer screening programs.[15,16,17,2]

## Carcinogenesis and early cancer interception

Early lung carcinogenesis is a complex process involving the sequential accumulation of molecular and genetic abnormalities. Putative pre-invasive lesions have been identified for NSCLC: squamous dysplasia and carcinoma in situ as precursors of lung squamous cell carcinomas (LUSC), and atypical adenomatous hyperplasia for lung adenocarcinomas (LUAD).[18]

### Risk factors

The dominant risk factor associated with lung cancer is tobacco exposure, with over two thirds of cases attributable to smoking.[19] With the rising incidence of lung cancer in non-smokers, additional risk factors have emerged including exposure to ionizing radiation (e.g. radon), occupational carcinogens (e.g. asbestos), and air pollution.[20] While mutagenic potential is a common feature amongst these exposures along with accumulating age-related mutations, chronic inflammation is likely also a critical component for lung cancer development and progression, as increased risk has been observed in chronic obstructive pulmonary disease and studies of particulate matter air pollution.[21,22] At the same time, adaptive immune surveillance likely limits cancer development via recognition of mutations as foreign neo-antigens, evidenced for instance by increased lung cancer incidence in immune-suppressed populations and immunogenetic associations with cancer risk.[23,24] As mentioned, family history is also an important risk factor. Taken together, these factors likely contribute in concert to influence lung cancer risk, but greater mechanistic understanding will provide further insights for improved cancer screening and prevention.

### Squamous carcinogenesis

Lung squamous cell carcinoma (LUSC) accounts for 30–40% of lung cancers worldwide and its pathogenesis is significantly linked to tobacco smoking.[25] In North America, LUSC prevalence has dropped to ~20%, with more LUSC occurring in peripheral lung, in association with a decline in smoking.[18] Central LUSCs are proceeded by preneoplastic bronchial changes ranging from hyperplasia and metaplasia to mild, moderate, and severe dysplasia, culminating in carcinoma in situ (CIS) (Fig.1). Preinvasive lesions including dysplasia and CIS typically persist or progress locally within the bronchial tree and are often linked to the development of invasive cancer in distant lung areas, supporting the concept of field cancerization. Recent molecular studies have revealed significant chromosomal instability and methylation changes in bronchial preneoplastic lesions, leading to increased expression of cell cycle pathways and DNA damage response genes, along with transitory metabolic reprogramming and immune sensing and unleashing of tissue resident immune cells. In high-grade pre-invasive lesions, the activation and mobilization of immune cells

of both innate and adaptive immunity develop. However, a concomitant inhibition of immune response occurs before invasion[26] and is associated with the risk of progression.[27] Even in CIS lesions that regress spontaneously, genomic, epigenomic, and transcriptomic characteristics resembling advanced invasive LUSC are observed.[28] This process involves the simultaneous expression of immune checkpoint molecules and suppressive interleukins, raising hope for the possibility of intercepting progression to invasive cancer by inhibiting the blocking factors of anti-tumor immune response.

## Adenocarcinoma carcinogenesis

Atypical adenomatous hyperplasia (AAH) is recognized as the only precursor of LUAD.[29] AAH may progress through preinvasive adenocarcinoma in situ (AIS), minimally invasive adenocarcinoma (MIA), and culminating in fully invasive LUAD (Fig.1).[30,31,32,33] LUAD precursors present radiologically as ground-glass opacity (GGO) predominant pulmonary nodules. The detection of these nodules has increased due to the widespread adoption of low dose computed tomography (CT) in lung cancer screening and the use of high-resolution CT scans for various medical purposes.[34] Over the past decade, extensive analyses of AAH, AIS and MIA have revealed a progressive increase in the complexity of their molecular evolution and associated immune responses,[35,36,37,38] with an increase in total mutation burden, copy number variant burden and cancer gene mutations in later-stage lesions.[35,36,37,38] Canonical cancer gene mutations such as *KRAS, EGFR*, and *BRAF* appear to be early genomic events during LUAD carcinogenesis,[35,36,37,38] in parallel with a gradual suppression of immunity, indicating ongoing “immunoediting”.[37,38,39] Preclinical studies demonstrated that reprogramming the TME may promote anti-tumor immunity and reduce the burden of invasive adenocarcinoma in mouse models.[40] More recently, single-cell technologies have revealed the complexity of tumor evolution with unparalleled resolution and have pinpointed alveolar type 2 (AT2)-like cells as contributors to LUAD progression.[41, 42] How the spatial context within the TME changes during different stages of disease progression remains to be clarified.

## Biomarkers of early detection

Small nodules in lung parenchyma can be efficiently detected by low-dose CT to allow early diagnosis of lung cancer and reduction of lung cancer mortality.[43] Recent advances in this field included the application of radiomics and artificial intelligence (AI) to develop non-invasive biomarkers for lung cancer risk prediction, early detection and prognosis prediction.[44] Analysis of the airway transcriptome of minimally invasive collected bronchial and nasal epithelial cells has proven effective for early diagnosis of lung cancer.[45] Likewise, the detection and molecular profiling of circulating tumor cells (CTC) in blood has been the objective of many studies to identify CTC biomarkers, although their application in lung cancer screening was questioned due to reported low sensitivity in prospective studies.[46,47] Cell-free nucleic acids (DNA and RNA), which can be passively and/or actively released by tumor cells and by other cells in the TME[48] have been the focus of many studies for early diagnosis, prognosis and prediction of therapy response. Low-pass whole genome sequencing of cell-free DNA (cfDNA) fragmentation profiles with the aid of AI has shown promising results in asymptomatic NSCLC.[49] Indeed, changes to the genomic and epigenetic architecture of cells during carcinogenesis result in a varied cfDNA

"fragmentome" in the peripheral blood.[50] Circulating cell-free microRNA in serum or plasma has also been investigated as a diagnostic tool and was validated in lung cancer screening trials.[51, 52] More recently, a comprehensive proteomic analysis in plasma samples has provided biomarkers for early prediction of lung cancer.[53] Finally, the integration of multiple types of biomarkers (e.g. nucleic acids and proteins) has also been attempted to detect various cancer types, including lung cancer. While the specificity appeared high (>99%), the sensitivity was low (~20–30%), limiting its application as a first-line diagnostic tool in high-risk subjects.[54]

## Tumor heterogeneity

Heterogeneity is an inherent characteristic of all cancers, including NSCLC. It may refer to differences among tumors from different patients, known as intertumoral heterogeneity, which reflect distinct carcinogenic pathways in each tumor. Alternatively, it can describe variation within a single tumor, termed intratumoral heterogeneity (ITH). The latter involves the presence of multiple and diverse clonal cancer cell populations within a single tumor. This diversity underpins the evolution and remarkable adaptability of tumors to various environments, which may lead to progressive invasiveness and aggressiveness, contributing to treatment resistance. A greater understanding of ITH in NSCLC from molecular and histopathologic perspectives is cumulatively impacting patient clinical management (Fig.2).

### Intratumor molecular heterogeneity

The coexistence of molecular and phenotypically distinct subclones within tumors are thought to develop under the so-called branched model of tumor evolution.[55] According to this model, tumors arise from a common ancestor eventually diverging and proliferating simultaneously, each with varying levels of efficacy. The punctuated model is a variant of the latter shaped by a macroevolution in which, after an extended stable period, many genomic aberrations occur within short bursts of time. Some tumors may evolve according to a neutral evolution model, in which cancer-driving alterations accumulate randomly within the cancer cell population without having a functional role in promoting tumor growth[56].

TRAcking Cancer Evolution through therapy [Rx] (TRACERx) is a prospective study which sought to define the role of genetic ITH on patient survival and disease characteristics.[57,58] Inference of the clonal architecture in the tumors in the study demonstrated that cancer driver mutations are under positive selection, not only when they occur as truncal mutations, but also when present in tumor subclones.[59,60] In the same study, a higher subclonal somatic copy number alteration (SCNA) burden, but not mutational burden, was associated with worse disease-free survival.[59] Additional biological consequences of genetic ITH include increasing neoantigen burden and subsequent immune response,[61] in addition to the generation of drug resistance mutations.[62]

Epigenetic alterations (e.g. DNA methylation and histone modifications) are crucial for tumor development and contribute to ITH. The most studied epigenetic deregulation in the context of ITH is DNA methylation. Extensive ITH in DNA promoter hypermethylation, driven by changes in DNA copy number, was found to contribute to the molecular and

phenotypic heterogeneity of LUAD and of lymph node metastases.[63] Further, genomic and DNA methylation changes appear to follow similar ITH evolutionary trajectories with a strong impact in cancer genes and pathways.[64] Given that drug development targeting epigenetic factors is underway, a deep understanding of epigenetic alterations in NSCLC will be essential for their effective implementation in the clinics.[65]

ITH can also be observed at gene expression and protein levels, which does not always originate from genomic ITH.[66] Transcriptomic diversity has been found between primary tumors and metastasis and contributes to tumor progression.[66,67] While multi-region sequencing provided foundational insights into ITH,[68,35] advancements in single cell sequencing and spatial transcriptomics have elevated ITH research to a new level and have provided comprehensive datasets.[69]

Intratumor spatial distribution of immune related proteins and patterns of proteins functionally involved in cell adhesion or endothelial cell interaction have been observed, some of which may be prognostic.[70,71] Deciphering the link between the static cancer genotype and its dynamic transcription and protein expression offers novel multilayers insights on the mechanisms of cancer adaptation.[72,73] The development of effective strategies to integrate ITH at the genomic, transcriptomic, and proteomic levels may provide opportunities for enhanced dynamic biological insights and the identification of novel therapeutic targets in lung cancer.[74,75]

## Histologic heterogeneity

The current World Health Organization (WHO) classification of lung cancers includes more than 20 entities, each defined by the unique histopathological appearances of the tumor cells and their growth pattern.[76] Morphological ITH is best observed in LUADs where the relative abundance of the different patterns of growth (lepidic, acinar, papillary, micropapillary and solid) is prognostic (Fig. 3).[77,78] Lepidic patterns are considered *in situ* tumors and thus associate with good prognosis [79] while non-mucinous LUADs with predominantly micropapillary and solid growth patterns are associated with poor prognosis.[80] Based on the IASLC grading system, any tumor with predominant or ≥20% of solid, micropapillary and complex glandular patterns are considered grade 3 (high grade).[81] Importantly, previous gene-expression based subtypes of LUADs [82] have not been correlated with the morphologic classification, possibly suggesting more complex multi-omics determinant of tumor growth patterns. The molecular mechanisms underlying morphological features of LUAD remain largely unknown, as do the molecular features associated with the development of lung cancers of mixed types.[83] A recent study employing micro-dissection of tumor regions in 19 LUADs with mixed histologic patterns or subtypes suggested an association of specific transcriptomic pathways with the different morphologies.[84] Other studies have reported that high-grade histologic patterns are associated with increased chromosomal complexity, higher burden of genomic aberrations and lower clonal diversity.[85,86]

## Clinical impact of tumor heterogeneity

Intertumoral heterogeneity and ITH also represent a major hurdle in the effectiveness of therapeutic strategies to treat lung cancer. Regarding intertumoral heterogeneity, even

within a defined histopathology, co-mutations and molecular differences among tumors can profoundly influence prognosis, tumor plasticity and therapeutic sensitivity.[87,88,89] The concomitant *RB1* inactivation in *EGFR*-mutant LUADs may facilitate histopathological transformation in response to targeted therapy[88] and *TP53* mutations cooccurring with *EGFR* are more likely to induce mixed responses.[90] Similarly, *STK11* mutations are linked to reduced sensitivity to immune checkpoint inhibitors (ICIs) in *KRAS*-driven LUAD,[91] while mutations in *KEAP1/NFE2L2* decrease sensitivity to different treatments and are associated with poorer prognosis.[92] These data highlight the importance of determining molecular clonal heterogeneity in otherwise uniform NSCLC populations.

Radiomics has recently opened avenues for better imaging assessment of intertumoral heterogeneity. The visualization and assessment of the whole tumor as well as the metastatic sites for radiomic features using machine learning may become an effective prognostic tool for prediction of response to immunotherapies.[93] Radiomic data may also lead to opportunities to escalate or change treatment or consider multimodality treatment options.

Similarly, cfDNA has been used as another non-invasive alternative for tracking molecular heterogeneity and determining clinical management. Analysis of cfDNA in the TRACERx cohort over multiple timepoints showed that about 40% of patients had tumors with ITH, in which a dominant clone emerged and replaced others between surgical resection of their primary tumor and disease recurrence.[94]

## Tumor cell plasticity

Cellular lineage plasticity (LP) refers to the ability of cells to switch phenotypes to distinct developmental lineages; it is integral to processes such as embryogenesis, tissue repair, and homeostasis.[95] However, cancer cells may hijack these mechanisms to adapt to stimuli, or aid tumor progression and metastasis. LP is increasingly recognized as a resistance mechanism to targeted therapies in lung cancer. Notable lineage states in LUAD include cancer stem cell phenotypes, epithelial-mesenchymal transition states, histologic transformation to neuroendocrine characteristics often with small cell lung cancer (SCLC) histology, and transformation to LUSC phenotypes. The mechanisms of LP in lung cancer are still poorly understood, which pose therapeutic challenges in the management of NSCLC at high-risk of LP.

While there are distinctions between various forms of plasticity, such as EMT or adenoneuroendocrine and adeno-squamous transformation, there is substantial overlap in the features and mediators of these phenomena. These changes are generally associated with hybrid metastable states characterized by elevated cellular plasticity and stem-like features. Single cell RNA sequencing in a genetically engineered mouse models (GEMM) of LUAD demonstrated that longitudinal transcriptional heterogeneity with disease progression was stereotypic and reproducible.[96] Certain factors that govern cell fate decisions during development and organ formation have resurfaced in cancer biology as key regulators of ITH and LP. Lineage tracing studies have played a crucial role in mapping the connections between progenitor and differentiated cells, uncovering varying degrees of plasticity across different stages of differentiation. The presence of these cell states may predict for poorer survival and are more resistant to chemotherapy[97] and immunotherapy.[98]

## Histologic transformation

The histologic transformation of LUAD to SCLC or LUSC, with or without therapeutic pressure, epitomizes the LP observed in lung cancer.[99] This transformation process likely involves a complex interplay of genetic, transcriptomic, epigenetic and immune factors.[100,101] The cell of origin, such as AT2 cells for LUAD, may play a key role in determining the predilection for certain oncogenic pathways. The bi-allelic inactivation of key tumor suppressor genes, *TP53* and *RB1*, is associated with histological transformation from LUAD to SCLC in tumors under tyrosine kinase inhibitors (TKIs) anti EGFR therapy.[102] A 'third hit' such as FGF9 upregulation is associated with SCLC trans-differentiation.[99] However, the exact mechanisms remain unknown. Recently, through GEMM and single-cell RNA sequencing, the upregulation of transcriptional programs induced by expression of *Myc* in cooperation with *RB1* loss have been implicated.[96] In addition, an intermediate basal stem-like cell state induced by EGFR inhibition may facilitate a tolerance to Myc-driven histological transformation. Histological transformation of LUAD to LUSC, whereby de novo deficient *Stk11* triggers extracellular matrix remodelling and p63 upregulation,[103] may similarly be characterized by an intermediate cell state with predisposing genetic alterations governing lineage plasticity in response to therapy. In response to *KRAS* targeted therapy, concurrent *STK11* mutations may alter the regulation of chromatin accessibility. Consequently, modulation of lineage-related transcription factors and the ELF5-ΔNp63 axis may drive histological transformation.[104] Lastly, cell-extrinsic factors may also play a crucial role in shaping lineage cell states by providing a permissive microenvironment for histological transformation,[105,106] although this remains underexplored to date.

## Drug-tolerant persister cells

Residual cancer cells that persist during treatment serve as the reservoir for the emergence of drug-resistance. This has prompted research into the cellular and molecular basis of drug-tolerance, the evolution of drug tolerant persister (DTP) cells and the identification of therapeutic vulnerabilities to target them, mostly in the context of targeted therapies. In *EGFR*-driven LUAD patient-derived xenograft (PDX) models, the transcriptional profile of DTP cells to TKIs has been identified in subpopulations of treatment-naïve tumors suggesting that, in some cases, these cells are present prior to treatment.[107,108] A role for epigenetic processes in maintaining the drug-tolerant state, including alterations in histone methylation patterns linked to repression of long interspersed repeat element 1 (LINE-1) elements was uncovered.[109, 110] Additional mechanisms that lead to activation of NFkB and JAK-STAT pathways, the antioxidant KEAP1-NRF2 pathway, EMT, senescence, translation reprogramming, impaired cell death and alveolar regeneration contribute to reduced treatment sensitivity in DTP and constitute a common mechanism by which lung cancer cells withstand therapy-induced stress.[111,112,113,114,115,92,116,107,117] Understanding when specific mechanisms of drug tolerance occur and how they can be targeted will be necessary to identify new approaches to mitigate or forestall the emergence of resistance.[118,119,120,121]

### Epithelial–mesenchymal transition (EMT)

EMT is a highly dynamic and reversible cellular program in which epithelial cells lose cell–cell junctions, display altered apical–basal polarity and acquire increased migratory capacity.[122] EMT histologically may manifest in poorly differentiated NSCLC or sarcomatoid/spindle cell carcinoma. Recent efforts have contributed to uncovering the molecular mechanisms associated with EMT-mediated resistance. ZEB1 directly binds the promoter of BIM, a positive regulator of apoptosis, repressing its transcription.[123] Similarly, Aurora kinase (AURK) B mitigates BIM-induced apoptosis to promote cell survival to treatment.[124] Activation of the YAP/FOXM1 axis promotes increased abundance of spindle assembly checkpoint effectors and mediates EMT-associated EGFR inhibitor resistance.[125] Concordantly, model systems of osimertinib resistance caused by EMT display activation of ATR-CHK1-AURKB signaling.[124] EMT-mediated resistance has also been attributed to the reactivation of downstream signaling pathways, including sustained activation of AXL,[126, 127,128] FGFR1[129,130] and SRC.[131] More recently CD70, which is highly expressed in NSCLC with mesenchymal phenotype,[132] was reported to be a targetable mechanism of EMT-associated EGFR inhibitor resistance.[133] In depth molecular characterization of baseline, on-treatment and resistant tumors and circulating tumor cells, together with functional analyses, might also help capturing the diversity and dynamics of EMT and contribute to uncovering the molecular alterations underlying its role in resistance to therapy.[122] These findings may reveal targetable dependencies and therapeutic combination rationales to re-sensitize mesenchymal lung cancers to targeted therapy in preclinical models.[123,124,130,127,129,133]

## Tumor microenvironment

Tumors, including NSCLC, consist of a diverse ecosystem of cells which, in addition to cancer cells, include a plethora of host cells with widely different and often opposing functions in oncogenesis. The importance of the immune cell contexture in NSCLC has been well established and draws from studies showing the TME to be strongly prognostic and the marked success of cancer immunotherapy increasingly becoming part of first-line care.[134,75,135] Many of the principles governing the immune- and cancer cell interrelationship seem to be well preserved across NSCLC stages, histological and molecular subtypes with certain exceptions, further bolstering the broad use of immunotherapy clinically. However, durable benefits to cancer immunotherapy occur in a minority of NSCLC patients for reasons still incompletely understood, warranting a better understanding of tumor-immune cell dynamics in NSCLC to spark a new wave of effective therapeutic strategies resulting in long-lasting tumor control.

### NSCLC immunogenicity and T-cell recognition

Intratumoral immune cells sculpt NSCLC genomic heterogeneity and vice versa, NSCLC cells influence immune cells through various mechanisms. Earlier findings in preclinical models showed increased tumorigenesis in the absence of key immune cells or anti-tumor effector molecules. This led to the understanding that tumors are constantly surveilled by cytotoxic T- and NK cells with immunogenic tumor cell clones being lysed in a process called ‘immuno-editing’. In the last decade, increasing evidence points to a role for T cells

in shaping tumor cell heterogeneity in NSCLC patients. Seminal studies including those from the TRACERx consortium have shown increased T-cell infiltration in patient tumors with high tumor-mutation burden (TMB), tumor areas exhibiting highly clonal neo-antigen expression, as opposed to low neo-antigen and clonally heterogenous tumor areas.[136,61] These cells, however, are found to be dysfunctional bearing an 'exhausted' phenotype characterized by the expression of multiple immune-regulatory molecules including LAG-3 and PD-1.[61,137] Anti-PD-1 was especially effective in these patients and to a lesser extent in those with a more T-cell and neo-antigen devoid TME.[136,61,137,138] Latter studies further showed compelling evidence of T-cells driving genomic tumor evolution, demonstrating decreased neo-antigen recognition potential in high T-cell infiltrated tumor regions through genomic, transcriptional and epigenetic mechanisms inducing immune-evasion (e.g. loss of HLA-alleles).[139,140,141] Whereas in established tumors immune-evasion leads to further tumor outgrowth, earlier CIS lesion in LUSC that spontaneously regressed showed increased T-cell presence and inflammation.[142] A significant proportion of CIS, however, still progresses to overt NSCLC exemplifying that immune evasion is a critical step early in tumorigenesis (Fig.4).[26,142] These studies highlight the impact of T-cells and other immune cells in driving ITH following the recognition and killing of immunogenic tumor cells. This process in turn can be reinvigorated by ICI leading to potentially long-lasting and even complete responses in a subset of patients. Further research is needed to understand what determines tumor behavior, response to ICI, and to learn from cells in and outside the TME to further develop improved biomarkers and therapeutic strategies.

## T-cell, myeloid and stromal cell heterogeneity in the TME and systemic anti-tumor immunity

NSCLC tumors can be roughly separated into T-cell inflamed tumors co-occurring with several different myeloid cells (e.g. dendritic cells, macrophages) and B-cells, and T-cell deserted or excluded tumors.[143,75,135,144]. The latter TME-contexture is more often seen in tumors from non/light-smokers, characterized by low TMB, loss of antigen-presentation machinery, strong driver mutations (e.g. EGFR and ALK) and can co-exist with a predominant neutrophilic infiltrate and/or stroma-rich regions with specific fibroblast-subsets mechanically impeding T-cell infiltration.[145,146,147,148,135] However, not all intratumoral T-cells are indeed tumor-specific; varying but significant amounts of T-cells in the TME recognize viral antigens or other cancer-irrelevant peptides.[149,150,151] Cancer-cell intrinsic mutations (e.g. in EGFR, STK11, genes involved in cell metabolism and increased TMB resulting in high neo-antigen load) and –extrinsic features (e.g. vasculature and associated hypoxia) are considered cardinal drivers of TME heterogeneity impacting the resultant anti-tumor immune response.[152] Other oncogenes and tumor suppressor genes are known to affect the immune response. For example, MYC oncogenic activation can prevent an appropriate response to interferon-gamma (IFN$\gamma$).[153] Tumor-specific genetic alterations that impact the processing and function of the HLA-I complex (e.g., B2M, TAP1) or the response to IFNy (e.g., JAK2, IFNGR1) are also common in lung cancer, influencing the composition of the TME[147,154] and impairing the response to ICIs.[146]

Crucial for induction of anti-tumor T-cells and efficacy of ICI is effective priming in tumor-draining lymph nodes (TDLNs) by dendritic cells presenting tumor antigens [155,156]. Priming

is an ongoing, dynamic process allowing for the continuous seeding and replenishment of T-cells also in response to ICI.[157,150] The importance of this process extends beyond immunity to the primary tumor and involves a systemic immune surveillance program potentially limiting the occurrence of distant metastasis.[158,159] Importantly, the initiation and strength of this systemic anti-tumor immune response can be severely hampered by local immune suppression in TDLNs by e.g. regulatory Tregs[160,161] or dendritic cells.[162] Moreover, TDLN-like structures in the TME called mature tertiary lymphoid structures (TLS) are also identified in NSCLC and correlate with an immune inflammatory environment and favorable prognosis, potentially by regulating T-cell attraction and maturation in concert with other perivascular niches.[163,164] Future strategies aimed at sensitizing NSCLC patients, and prolonging responses to ICI, will have to consider aspects regarding systemic anti-tumor immunity.

### Translational aspects to ICI resistance

Contrary to earlier beliefs, the response to ICI in NSCLC seems to be not only dependent on activation of pre-existing tumor T-cells but relies on a replenishment by circulating T-cells including T-cells with early differentiation phenotypes and novel TCR-specificities.[157, 158, 159] Primary resistance to ICI can occur at multiple steps in anti-tumor immunity initiation ranging from a paucity of immunogenic tumor antigens, antigen presentation, strong immune suppression or high stromal/vascular impediment, as highlighted earlier. A significant proportion of patients, however, respond initially but eventually progress in a process called secondary immune resistance. Tumor behavior in secondary resistance shares similarities to primary immune resistance, but there are important differences as well including the frequent occurrence of oligoprogression (progression of a single lesion, with persistent immune control of others) and related to this, better survival as compared to primary resistance.[165] Genomic and proteomic characterization of the TME of progressing lesions has identified similar phenomena as observed in primary immune resistance relating to impaired immunorecognition or antigen presentation contribute to immune evasion (e.g. decreased expression of the HLA-protein complex,[166, 146] loss of clonal neo-antigens and acquisition/selection of mutations associated with ICI-resistance (e.g. in *STK11*).[91,167,165] Additionally, chronic IFN signaling in response to ICI may paradoxically induce PD-L1-independent routes of immune suppression through epigenetic alterations induce inhibitor ligand and molecule expression capable of suppressing more exhaustion-sensitive $CD8^+$ T-cells.[165,168] Interestingly, blocking (chronic) IFN-signaling with JAK-inhibitors has shown potential together with ICI in NSCLC.[169] These studies and others highlight the importance of comprehensive multi-omic and translational studies of NSCLC resistant to ICI to develop rational immunotherapy combinations.

## Metabolic Landscape of Lung Cancer

The metabolic reprogramming of NSCLC has been studied in recent years with the aim of identifying vulnerabilities that could be translated into novel therapeutic approaches (Fig.5). Several studies have highlighted the role of fatty acid, glucose, and mitochondrial metabolisms in NSCLC tumorigenesis. The RAS pathway is important in metabolic rewiring and potentially targetable in NSCLC. KRAS-mutant NSCLCs with co-mutations in STK11

and KEAP1 represent a distinct subset of the disease with unique characteristics and treatment challenges.[170, 171]

In NSCLC, glutathione, an essential antioxidant in cells, and associated enzymes are often not adequately regulated.[172,173] This promotes resistance to oxidative stress and helps cancer cells survive and multiply.[173] STK11 is widely studied as the kinase that activates AMPK to rewire metabolism by modulating mTOR signaling.[174,175] Recent studies have shown that the salt-inducible kinase (SIK) family members (SIK1 and SIK3) mediate the tumor suppressor role of STK11 in NSCLC but not AMPK.[176,177] KEAP1 is a key regulator of the cellular antioxidant response. It controls the activity of NRF2, a transcription factor that regulates gene expression in antioxidant and detoxification processes. The KEAP1-NRF2 pathway regulates the expression of antioxidant genes, which promotes cancer cell survival and resistance to therapy.[178] The interplay between glutathione and the KEAP1-NRF2 pathway in NSCLC underscores the importance of understanding redox regulation in cancer progression and treatment. KRAS-mutant NSCLC with co-mutations in STK11 and KEAP1 upregulates ferroptosis evasion pathways. Targeting ferroptosis evasion by inhibiting stearoyl CoA desaturase 1 (SCD1), a master regulator of fatty acid metabolism, has shown promise in preclinical models.[171] SCD1 can directly regulate the SIK1, which disrupts the multimodal ferroptosis protection of STK11/KEAP1 co-mutated NSCLC cells.[171,179,177] Studies have shown that the SIK1-NRF2 axis can modulate the sensitivity of STK11/KEAP1 co-mutant cancer cells to ferroptotic cell death and chemotherapy [176,177]. SIK1 also controls the serine glycine one-carbon metabolism (SGOC), which plays a crucial role in nucleotide synthesis, methylation reactions, and redox balance within the cell. Most interestingly, SIK1 was found to negatively regulate the SGOC in STK11/KEAP1 NSCLCs by activating SHMT, which increases the cellular antioxidant mechanism.[180] Therefore, further exploration of the interplay between SIK1, NRF2, and the STK11/KEAP1 co-mutant genotype holds promise for identifying potential therapeutic targets and developing personalized treatment strategies for patients with oncogene-driven subsets of NSCLC.

Metabolic reprogramming may facilitate the formation of an immunosuppressive microenvironment by tumor cells, where lactic acid, a metabolite of glycolysis, is now documented to contribute to this process.[181,182] Lactic acid can directly suppress the TME by inducing apoptosis of natural killer cells (NK) and natural killer T-cells (NKT).[183,184] In addition, it can inhibit the proliferation of cytotoxic lymphocytes,[185] block IFNγ and IL4 production by NKT cells, inhibit the differentiation of dendritic cells (DCs) and increase the production of IL10. Furthermore, lactic acid has been shown to confer changes in the innate and adaptive immune responses by driving the development of myeloid-derived suppressor cells.[186] Studies have demonstrated a positive correlation of high lactic acid concentrations in the TME with tumor invasion and metastasis in multiple cancer types including lung cancer.[187,188,189] As such, targeting tumor cell production and transport of lactic acid may provide an effective strategy for anti-tumor therapy.[190]

Considering the immunosuppressive effects of lactate metabolism in lung cancer and its effects on anti-tumor immunity, several signaling pathways and targets have been reported that may offer therapeutic targeting or inhibition. Monocarboxylate transporters [181,182,183,184] are lactic acid transporters[191] and the efflux of lactate from tumor cells

is mediated by monocarboxylate transporter 4 (MCT4), also known as SLC16A3, while SLC16A1 is thought to mediate its influx.[192] While the role of SLC16A3 in tumor development and lactate metabolism is not well understood, studies have reported that SLC16A3 is highly expressed in lung cancer[193] and is negatively correlated with immune cell infiltration. Pharmacological inhibition of SLC16A3 in tumor cells attenuated intercellular lactic acid exchange, resulting in the reduction of acidity within the TME and increased the efficacy of anti-PD-1 therapy, highlighting the potential targeting of lactate acid transporters in overcoming immunotherapy resistance.[181] A number of MCT1 inhibitors have been reported that have shown promising pre-clinical activity and include SR138009[194] and AZD3965.[195] The process of lactylation has recently emerged as an important form of protein modification where studies have demonstrated that lactate modulates cell metabolism in NSCLC through increased histone lactylation in the promoter regions of the HK-1 and IDH3G genes.[196]

Lactate has also been implicated as a messenger in the cross talk between cancer cells and immune cells, in particular, tumor-associated macrophages, where it has been shown to influence macrophage polarization.[197] Furthermore, the nuclear factor of activated T-cell c2 (NFATc2) promotes M2 macrophage polarization by regulating USP17 via transcriptional stimulation. Upon overexpression of USP17 in LUAD cell lines, lactate levels were upregulated, an effect that was reversed in response to inhibition of NFAT.[198]

More recently, there has been an emerging interest in the metabolic reprogramming of NSCLC by oncogenic driver mutations such as EGFR, EML4-ALK and BRAF.[199] Research examining the metabolic alterations in EGFR mutant NSCLC highlights the dependency of these tumors on glucose,[200]glutamine[201] and lactate metabolism,[202] in addition to fatty acid synthesis.[203] Compared to EGFR wild-type and EGFR-mutated NSCLC, patients with ALK rearrangements exhibit higher glucose metabolism, where targeting of EML4-ALK fusions with ceritinib in xenograft models demonstrates a reduction in glycolysis. It has been suggested that this process is mediated through the EML4-ALK-HIF1α-HK2 axis.[204] While few studies have been reported on the metabolic effects of BRAF mutations in NSCLC, it has been shown that BRAF V600E-driven tumors can maintain mitochondrial function and glutamine metabolism through their addiction to autophagy.[205] Furthermore, the failure of late stage Atg7-deficient tumors to thrive was a consequence of impaired mitochondrial function and limited availability of substrates such as glutamine, resulting in tumor crisis which was incompatible with tumor growth.[206]

Further studies to better understand the metabolic plasticity that exists between tumor cells and immune cells may provide new knowledge supporting the development of novel combination (metabolic and immune) therapies in lung cancer.

## Microbiome

Although the lungs were previously considered sterile organs, despite constantly being exposed to microorganisms present in the upper respiratory airways and in airborne bioaerosols, with the advent of NGS, a variety of lung commensal microbiota were identified.[207, 208] Lung microbiota interacts with the gut microbiota (Gut-Lung axis) by

means of ligands and metabolites, which might be pivotal to train the lung immune system and protect from exacerbated inflammatory responses to inhaled antigens.[209] This commensal microbiota tolerance develops early in the newborns (within ~2 months from birth)[210] and appears to play important roles in preventing inflammation-related lung damage as well as lung carcinogenesis.

### Microbiome in NSCLC initiation and progression

Dysregulation of lung microbiota might lead to changes in the pre-neoplastic/neoplastic lung microenvironment by reshaping tumor-infiltrating immune cells, ultimately contributing to cancer onset and progression.[211,212] This may be due to the role of chronic inflammation in lung carcinogenesis, with evidence that repeated exposure to antibiotics was associated to NSCLC risk [213,214]. Several bacteria have been associated with chronic inflammation and subsequent increased cancer risk including lung cancer, like *Mycobacterium tuberculosis*.[215] Liu *et al.*[216] showed that lung microbiota in patients with NSCLC are enriched in genus *Streptococcus* while depleted in genus *Staphylococcus*. It was also reported that genus *Thermus* was more abundant in patients with advanced NSCLC, while genus *Legionella* was more abundant in patients who develop metastases.[217] Ma et al. found that NSCLC patients had enrichment of Th1 and Th17 cells reacting to *Streptococcus salivarius* and *Streptococcus agalactiae* compared to healthy controls.[218]

### Microbiome in lung cancer therapies

The therapeutic efficacy of ICIs has been correlated with the composition of the microbiome, in combination with the effect on the host immune system. Routy *et al*[219] observed an increased relative abundance of *Akkermansia muciniphila* in anti-PD-1 responders compared to non-responders. However, taxonomic-centric microbiome studies have shown huge limitations, as different immune-regulatory strains with distinct taxonomic assignments within the same cancer types receiving the same treatment have been reported.[220] This discrepancy is likely due to the rapid evolution of bacteria at the strain level and the profound inter-individual heterogeneity of the human microbiota, in addition to differences in microbiota acquisition, processing and identification. Assessing the functional capacity of the microbiota across different patient cohorts using standardized approaches may serve as a better biomarker in this setting.

## Future perspectives

Basic and translational research in lung cancer during the last three decades has greatly advanced our understanding of the molecular pathogenesis and biology of lung cancer. Technological advances in high throughput nucleic acid sequencing and proteomic analysis, computational science, medicinal chemistry, molecular modeling, biobanking and large data science have accelerated these research achievements, with transformational impacts in the clinical detection, diagnosis, treatment and management of patients with lung cancer. Continued advances in technologies, including single cell multi-omics analyses and AI will continue to accelerate our research advances, raising further optimism on the future prospect of curing lung cancer in a larger number of patients. In addition, for these innovations to translate into meaningful clinical impact, integrating patient-derived samples, including pre-

and post-treatment, multi-region samples in well-annotated patient cohorts will not only refine our understanding of NSCLC biology but also accelerate the development of truly personalized treatment strategies, ensuring these advancements reach the patients who need them most.

In the field of early-stage lung cancer, efforts have focused on monitoring high-risk groups, but there is a need for solutions in biomarker screening for early detection, preventive treatments for cancer interception, and risk stratification to avoid overtreatment. Developing standardized immune biomarkers for pre-cancerous conditions is crucial. Additionally, integrating AI-assisted medical decision-making would provide guidance on clinical decisions within the disease's temporal context. Leveraging the latest research findings requires conducting prospective, multicentric clinical trials on biomarkers for detection, prediction, and interception. Although it has long been recognized that LUAD precursors often manifest as GGO-predominant lung nodules, and that severe dysplasia and CIS serve as LUSC precursors, efforts to intercept these lung cancer precursors have been limited by our rudimentary understanding of the molecular events and tumor microenvironment changes driving malignant transformation and neoplastic evolution. However, emerging evidence highlighting the simpler molecular landscape and more active immunity in lung cancer precursors has spurred interest in immune interception, aiming to leverage the enhanced immune activity in precancers to prevent the development of invasive lung cancers. In this context, multiple clinical trials that explore the efficacy of immunotherapies (NCT04789681, NCT03634241, NCT03347838) have been initiated and encouraging results would represent a significant step forward in immune interception for lung cancer prevention.

Tumor heterogeneity is a fundamental characteristic of neoplastic progression including invasion, tumor growth and metastasis. It also plays a role in response to initial therapy and the development of resistance mechanisms to therapy. The TRACERx studies have provided some clues to the nature and mechanism of tumor heterogeneity in lung cancer, yet these are likely to represent the tip of an iceberg, as to date, analyses have mainly been limited to exome, gene copy number and transcriptome levels. Further functional genomics analyses are also strongly recommended. Heterogeneity at the histological level is common involving all types of NSCLC, yet the molecular basis of such heterogeneity is poorly understood. Future studies will need to be ambitious and involve multiple 'omics analyses, single cell and spatial multi-omics analyses, as well as samples from primary versus metastatic tumors, and pre- versus post-treatment tumors. The incorporation of living patient-derived cell lines, organoids and xenografts also provides great benefits in the ability to model tumor biology with functional studies.

The development of resistance is almost a universal feature of systemic therapies in lung cancer, and despite extensive research, limited progress has been achieved in understanding the mechanisms and strategies to overcome them. In the field of targeted therapies, recent research interest has focused on the mechanisms of drug tolerance and plasticity, the latter involving histologic transformation from adenocarcinoma to neuroendocrine or squamous cell carcinoma. Future research to understand the mechanism of DTPs may lead to novel strategies to improve treatment efficacy and cure rates for these targeted

therapies. Understanding the mechanisms of plasticity may lead to new strategies to identify predictive biomarkers for their development and/or mitigate resistance-causing histologic transformation.

Advancing our understanding of lineage plasticity in lung cancer is critical for overcoming therapeutic resistance and improving outcomes. Future research must prioritize deciphering the molecular drivers of LP, such as epigenetic reprogramming, TME influences, and key transcriptional regulators like ZEB1 and YAP/FOXM1. Targeting EMT-associated dependencies, immune evasion pathways, and plastic cancer stem cells offers promising avenues for intervention. Integrating single-cell profiling and functional studies with clinical insights will drive personalized therapies addressing LP-driven resistance mechanisms.

A large subset of patients does not respond to currently approved or experimental ICIs, most likely due to limited tumor immunogenicity and T-cell presence in the TME. To improve response rates, future studies will have to shift from targeting pre-existing exhausted tumor-infiltrating T cells to strategies incorporating insights from early T-cell priming, homing and systemic immunosurveillance, to co-opting cells in the TME (e.g. macrophages, fibroblasts) or genetically modifying T cells as cellular therapies. Linking tumor cell biology (including oncogene-driven metabolic vulnerabilities, such as ferroptosis evasion, redox imbalance, and lactate generation) to immune contexture and downstream T-cell responses will be critical to reverse the T-cell hostile environment in NSCLC. Finally, whereas tumor vaccination seems of limited utility in the advanced stage disease setting, pivoting to earlier (including preinvasive) and neo-adjuvant stages together with ICI will be critical for durable memory and resulting clinical responses.

Current technologies enabling multi-omics analyses, combined with AI, have recently led to exciting discoveries in translational research. These advancements will further accelerate our knowledge and understanding of NSCLC biology, offering hope for less toxic and more personalized therapeutic strategies both in the frontline and at relapse. These innovations bring the promise of extended survival for many patients and the potential to increase the chance of cure for a greater number of patients.

This review is based on the knowledge of members of the IASLC Basic and Translational Research Committee, thus its scope may be limited by their expertise. In the future, additional more focused and in-depth reviews will be published by the committee.

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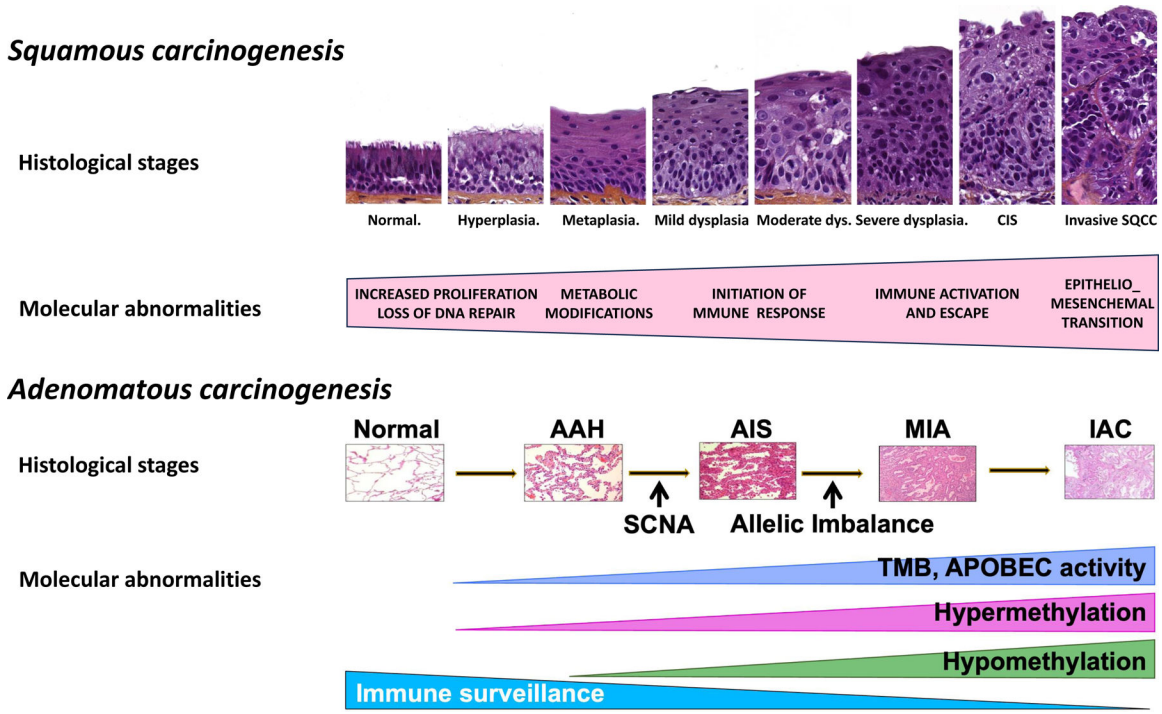
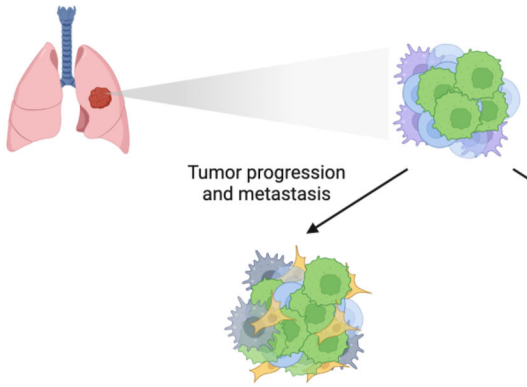



**Figure 1.**

Multi-stage lung carcinogenesis. The chronological histological changes observed in lung squamous and adenomatous carcinogenesis and their cumulative molecular changes. Abbreviations: Dys, dysplasia; CIS, carcinoma in situ; SQCC, squamous cell carcinoma; TMB: tumor mutation burden; SCNA: somatic copy number alteration; AAH: atypical adenomatous hyperplasia; AIS: adenocarcinoma in situ; MIA: minimally invasive adenocarcinoma; IAC invasive adenocarcinoma. Hypomethylation and Hypermethylation: these refer to aberrant methylation changes compared to matched normal lung tissues.




| | | |
|---|---|---|
| **Intratumor Heterogeneity** | | |
| GENETIC | *SMARCA4*, High ITH in SCNA | *RB1, STK11, KEAP1/NFE2L2* mutations<br>High ITH (antigen burden) |
| EPIGENETIC | High ITH in DNA methylation | |
| TRANSCRIPTIONAL<br>POST-TRANSCRIPTIONAL | Pro-proliferation, cell-cell interaction<br>Spatial organization of TME | Low IFN-γ signature |
| **Cell identity / Histological transformation** | | |
| GENETIC | *TP53* loss, *RB1* loss, *WGD, APOBEC* | WNT alterations |
| EPIGENETIC | EZH2, DNMT1, LSD1 | EZH2, DNMT1, LSD1 |
| TRANSCRIPTIONAL<br>POST-TRANSCRIPTIONAL | NOTCH suppression<br>Immune suppression | Lineage determining TF upregulation<br>AKT upregulation, DLL3 upregulation |
| **Cell identity / Epithelial-to-mesenchymal transition** | | |
| GENETIC | | YAP/FOXM1 |
| EPIGENETIC | microRNA-200/ZEB1 axis | YAP/TEAD |
| TRANSCRIPTIONAL<br>POST-TRANSCRIPTIONAL | ZEB1, E-cadherin loss | YAP/FOXM1, YAP/FAK, ASCL1, AXL,<br>FGFR1, Src, SAC, ZEB1 |
| **Tumor progression** | | |
| GENETIC | High TMB, High CNVs | |
| EPIGENETIC | | LINE-1 |
| TRANSCRIPTIONAL<br>POST-TRANSCRIPTIONAL | High-plasticity cell state<br>Alveolar type 2 cells (AT2)-like cells | Alveolar regeneration<br>ATR/CHK1/Aurora B, NF-κB, KEAP1-NRF2<br>CD70, IL-6 producing CAFs, JAK-STAT<br>Translation reprogramming |

**Figure 2.**

Molecular regulators and potential therapeutic targets of tumor heterogeneity and plasticity in lung cancer. Graphical representation of genetic, epigenetic, transcriptional and post-transcriptional mechanisms that promote tumor progression, metastasis and resistance to therapy. Abbreviations: SCNA: Somatic copy number alterations; ITH: Intratumor heterogeneity; TME: Tumor microenvironment; CNV: copy number variations; TMB: tumor mutational burden; SAC: Spindle assembly checkpoint proteins; CAFs: Cancer-associated fibroblasts.

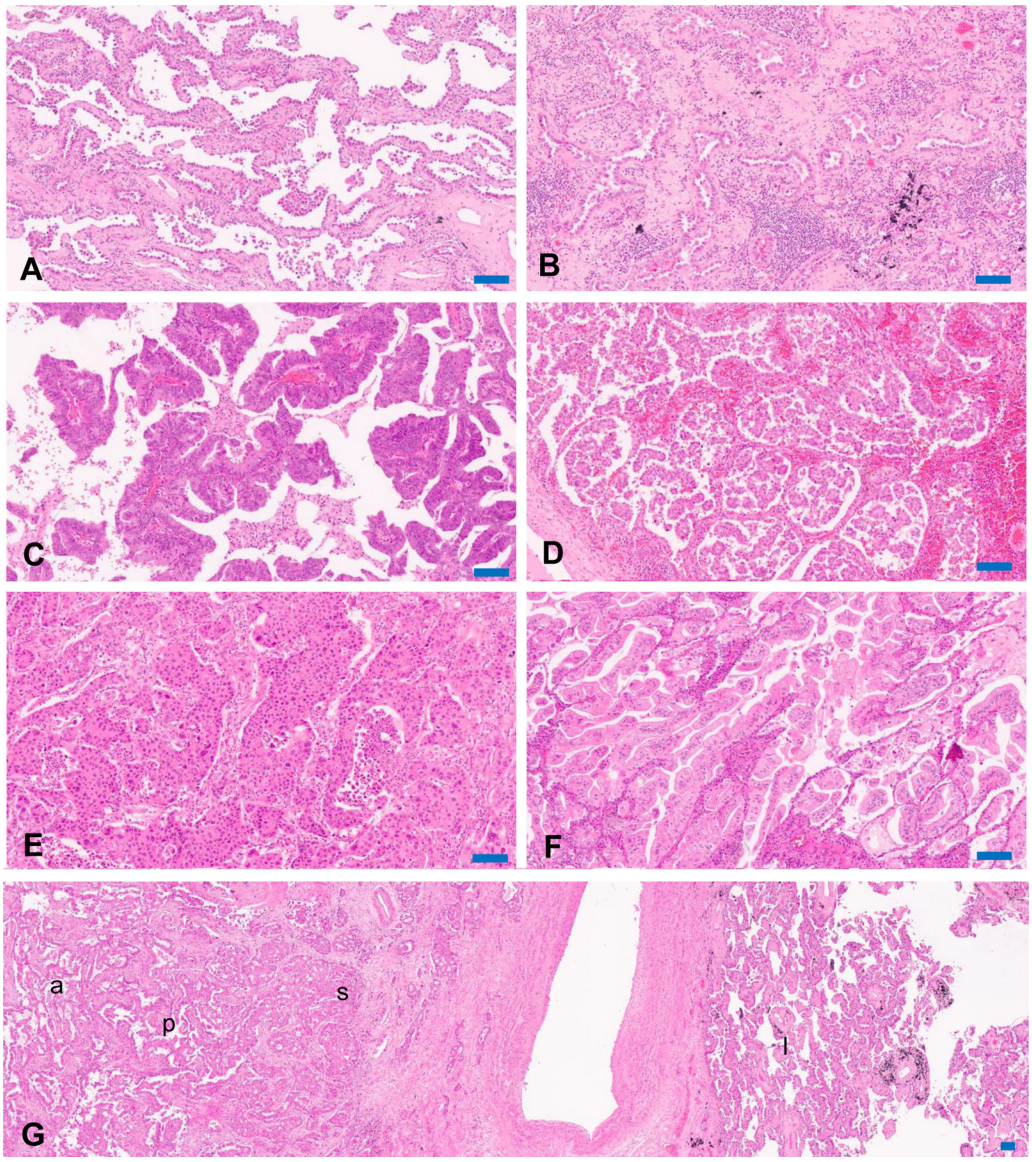



**Figure 3.**

Heterogeneous growth patterns observed in LUADs. (A) Lepidic pattern is considered in-situ non-invasive growth, while (B) acinar, (C) papillary, (D) micropapillary and (E) solid patterns are regarded as invasive growth patterns. (F) Mucinous adenocarcinoma is distinguished for its finely vacuolated cytoplasm that contains mucin substance. (G) Up to 80% of non-mucinous LUAD contains a mixture of multiple patterns. The predominant pattern is used to subtype non-mucinous LUAD, and their relative abundance is used for grading the tumor. Abbreviations: lep: lepidic; acn: acinar; pap: papillary; sol: solid. Scale bar: 100 μm

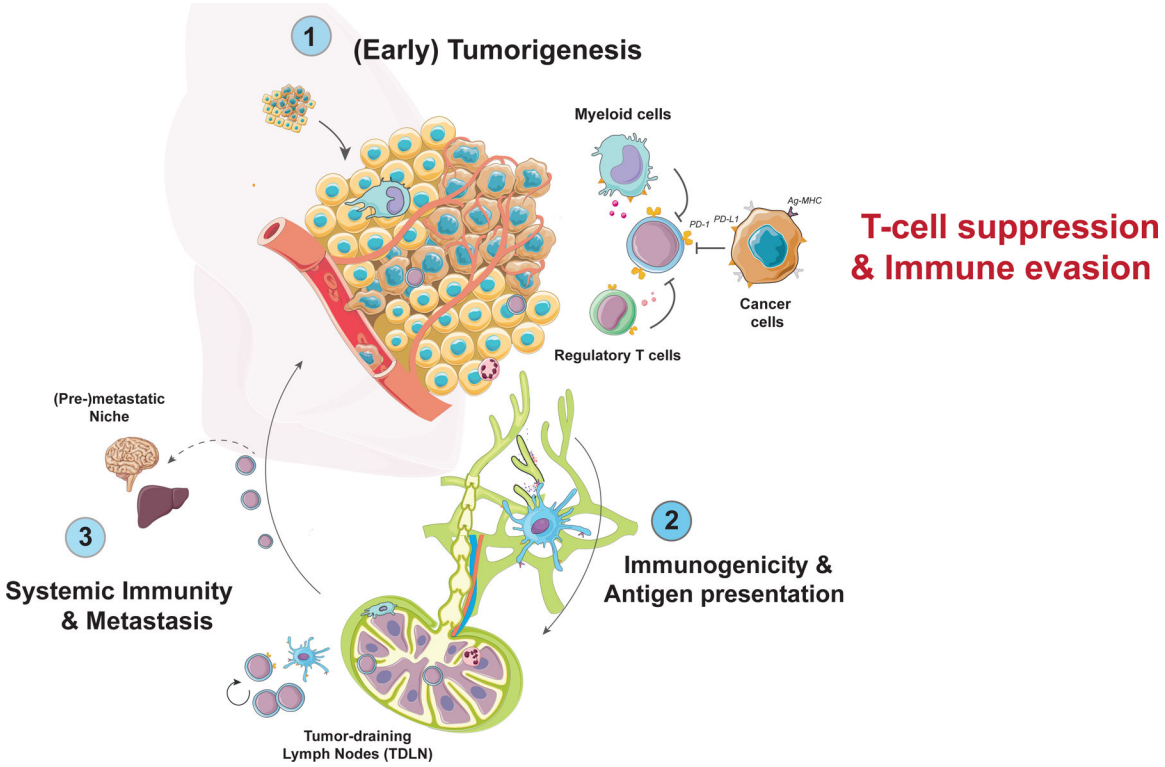



**Figure 4.**

Immune evasion as a hallmark of lung cancer progression. (1) Evasion of anti-tumor immunity occurs early during tumor progression in preneoplastic lesions through a plethora of different mechanisms including contact-dependent [e.g. PD-1/PD-L1 checkpoint interaction, loss of immunogenicity through MHC- or neo-antigen (Ag) loss)] or contact-independent processes (e.g. suppressive cytokines, metabolites etc.). (2) These mechanisms extend beyond the tumor microenvironment (TME) and affect antigen presentation in tumor-draining lymph nodes (TDLNs) and systemic anti-tumor immunity. (3) Furthermore, several immune cells either positively (e.g. circulating tumor-specific CD8+ T cells) or negatively (e.g. neutrophils) contribute to distant organ metastases through conditioning of the (pre) metastatic niche.

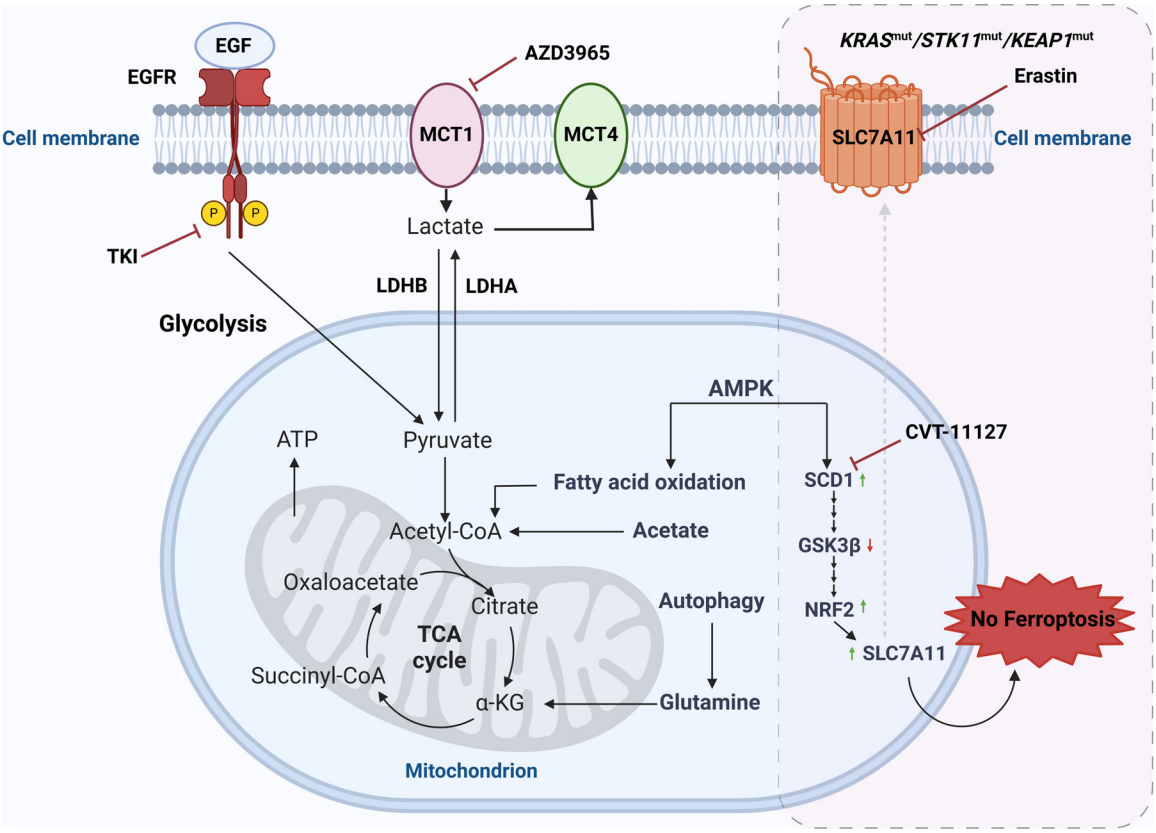



**Figure 5.**

Metabolic reprogramming in EGFR and KRAS driven NSCLC. EGFR-mutant NSCLC demonstrates a high dependency on glucose, glutamine and lactate metabolism, in addition to fatty acid synthesis. Monocarboxylate transporters are lactic acid transporters, which mediate the influx and efflux of lactate in tumor cells. MCT1 inhibitors (e.g. AZD3965) have preclinical activity in lung cancer cells by inhibition of the immunosuppressive effects driven by lactate metabolism. The interplay between STK11 and KEAP1 co-mutations in KRAS-mutant NSCLC can lead to rewiring of cellular metabolism and redox homeostasis dysregulation, influencing cancer progression and treatment response. Ferroptosis evasion is enriched in STK11 and KEAP1 co-mutated lung cancer cells, where several genes regulated by NRF2 encode proteins implicated in ferroptosis control, including SLC7A11, a key regulator of glutathione metabolism. Targeted inhibition of SCD1 activity by CVT-11127 can disrupt ferroptosis in STK11 and KEAP1 co-mutated cells. Further exploration of NSCLC metabolic plasticity may enable the strategic use of novel metabolic inhibitors, alone or in combination with immunotherapies, to overcome treatment resistance and enhance therapeutic efficacy.