

DNA Methylation in Lung Cancer: Predictive Biomarkers for Effective Immunotherapy

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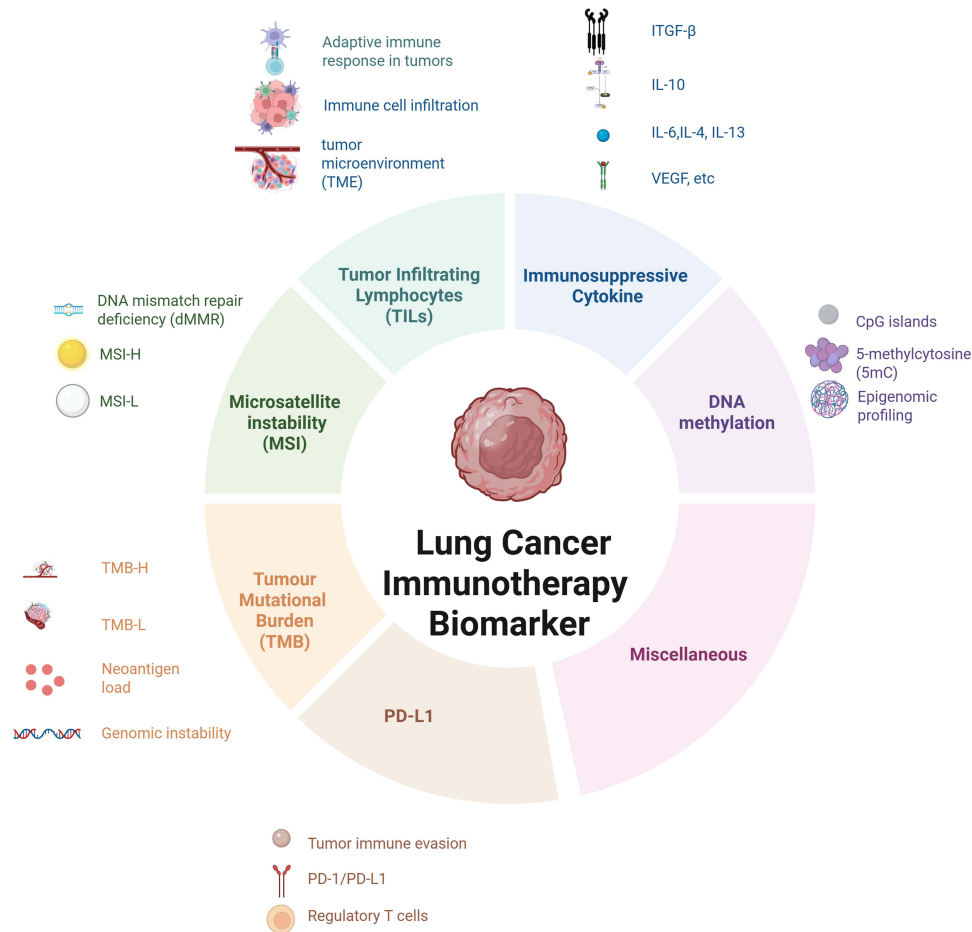
Abstract: Lung cancer remains a leading cause of cancer-related mortality worldwide, and immunotherapy has emerged as a promising treatment modality. However, its efficacy is limited to a subset of patients, necessitating predictive biomarkers for personalized treatment strategies. DNA methylation (DNAm) is increasingly recognized as a crucial regulator of gene expression and immune responses in the tumor microenvironment. This review focuses on DNAm as a key epigenetic biomarker for predicting and enhancing immunotherapy efficacy in lung cancer. We highlight DNAm changes in key immune-related genes and their association with tumor phenotype, immune cell infiltration, and response to immune checkpoint inhibitors (ICIs). The review also evaluates established and emerging genomic and non-genomic biomarkers, including tumor mutational burden (TMB), microsatellite instability (MSI), PD-L1 expression, tumor-infiltrating lymphocytes (TILs), and immunosuppressive cytokines in case of lung cancer immunotherapy. Furthermore, the potential for integrated epigenetic signatures and minimally invasive diagnostic approaches, such as liquid biopsies, is discussed. Finally, we address the challenges and future directions for translating epigenetic biomarkers into clinical practice to improve immunotherapy outcomes and reduce immune-related adverse events.

Keywords: tumor mutational burden, tumor microenvironment, epigenetic biomarkers, immunotherapy, microsatellite instability, tumor-infiltrating lymphocytes

Introduction

Lung cancer remains to be a predominant cause of cancer-related mortality globally, with an estimated 226,650 new cases and 124,730 deaths projected for 2025.^{1,2} Despite advancements in screening, diagnosis, and therapeutic interventions, overall survival rates remain suboptimal, particularly in advanced-stage disease. Challenges such as late detection, significant tumor heterogeneity, and frequent development of therapy resistance persistently hinder the clinical outcomes.³ Lung cancer originates from complex interactions between genetic mutations⁴, environmental exposures, and epigenetic modifications.⁵ DNA methylation (DNAm), which involves the addition of a methyl group to cytosine residues in CpG dinucleotides, is one of the most extensively investigated epigenetic mechanisms associated with disease.^{6,7} DNAm is crucial for regulating gene expression, maintaining genomic stability, and controlling key cellular processes. Aberrant DNAm patterns, including promoter hypermethylation of tumor suppressor genes, drive oncogenesis, facilitate immune evasion, and influence tumor progression.^{8,9} Immunotherapy has significantly transformed the treatment paradigm for advanced non-small cell lung cancer (NSCLC). Immune checkpoint inhibitors (ICIs) targeting pathways such as PD-1, PD-L1, and CTLA-4, including nivolumab, pembrolizumab, atezolizumab, and durvalumab, have demonstrated substantial and durable survival benefits.^{10–12} However, the therapeutic responses to these agents exhibit considerable variability. Although “hot” tumors with abundant immune infiltration respond favorably, “cold” tumors characterized by poor T cell infiltration often remain unresponsive.^{13–15} This heterogeneity underscores the urgent

Graphical Abstract



need for reliable biomarkers to predict which patients are most likely to benefit from ICIs therapy. Integrating DNAm profiling into immunotherapy is a promising strategy to address this challenge. Aberrant methylation of tumor suppressor genes, immune regulatory genes, and pathways involved in antigen processing and presentation can alter neoantigen load, modulate immune cell recruitment, and shape ICI sensitivity or resistance.^{16,17} Notably, DNAm signatures are stable, detectable early in tumorigenesis, and measurable in minimally invasive samples such as blood, sputum, and bronchial aspirates. Combining DNAm signatures with established biomarkers, such as PD-L1 expression, tumor mutational burden (TMB), microsatellite instability (MSI), and tumor-infiltrating lymphocytes (TILs), may enhance patient stratification and refine immunotherapy decision-making. In this review, the current understanding of DNAm as a predictive biomarker for lung cancer immunotherapy. DNAm mechanisms, their influence on immune regulation, and their utility for noninvasive detection. Also highlight evidence from recent clinical studies evaluating DNAm-based biomarkers for patient selection and treatment responses. By integrating emerging epigenetic and immunological insights, this review provides a framework for the clinical application of DNAm-driven biomarkers in precision immunotherapy for lung cancer.

DNA Methylation

Cancer development emerges from intricate interactions between genetic modifications, epigenetic shifts, and external factors.¹⁸ Epigenetic abnormalities, like alterations in DNAm, contribute to controlling genes and upholding genomic

stability. Genetic mutations and epigenetic variations, encompassing DNAm, histone modifications, and non-coding RNA, form the foundation of lung cancer onset.¹⁹

DNAm, a chemical alteration, involves adding a methyl group to the fifth carbon of cytosine, primarily at CpG sites. CpG islands are present in many genes, spanning both widely active and tissue-specific ones. Disruptions in this methylation process can initiate cancer formation, thus serving as a crucial marker for cancer therapies,²⁰ Figure 1. It illustrates the role of DNAm biomarkers in enhancing immunotherapy and enabling noninvasive diagnosis of lung cancer. The efficacy of immunotherapy in lung cancer is contingent on various factors. It shows the interplay between tumor immune phenotypes and epigenetic regulation, influencing the immunotherapy response in lung cancer. Tumors are classified as either “cold” or “hot” based on their immune activity. Cold tumors are characterized by low immune cell infiltration, limited antigen presentation, and weak inflammatory signaling, resulting in poor immunotherapy responsiveness. In contrast, hot tumors exhibit abundant infiltration of cytotoxic CD8+ T cells and natural killer (NK) cells, higher expression of immune checkpoints such as PD-1/PD-L1, and a more active immune microenvironment that favors effective immune response. Regulatory T cells (Tregs) and fibroblasts (including cancer-associated fibroblasts) contribute to immune suppression and extracellular matrix remodeling, supporting tumor progression. Epigenetic alterations, such as DNAm, play a central role in shaping these tumor phenotypes. DNA methyltransferases (DNMTs) catalyze the addition of methyl groups to cytosine residues, leading to promoter hypermethylation and transcriptional silencing of tumor suppressor and immune-related genes. Histone methyltransferases (HMTs) and histone deacetylases (HDACs) modify histone tails HMTs by adding methyl groups and HDACs by removing acetyl groups thereby altering chromatin conformation and gene accessibility. Mutations in the SWI/SNF (SWItch/Sucrose Non-Fermentable) chromatin-remodeling complex disrupt nucleosome positioning and impair immune-gene expression. Genome-wide hypomethylation can induce genomic instability and aberrant oncogene activation. These epigenetic changes collectively determine whether a tumor remains immune-cold or transitions to an immune-hot phenotype, influencing its sensitivity to immune checkpoint blockade therapies targeting PD-1/PD-L1. Therefore, DNAm signatures serve as promising epigenetic biomarkers for assessing tumor immune status and guiding immunotherapy strategies in patients with lung cancer.

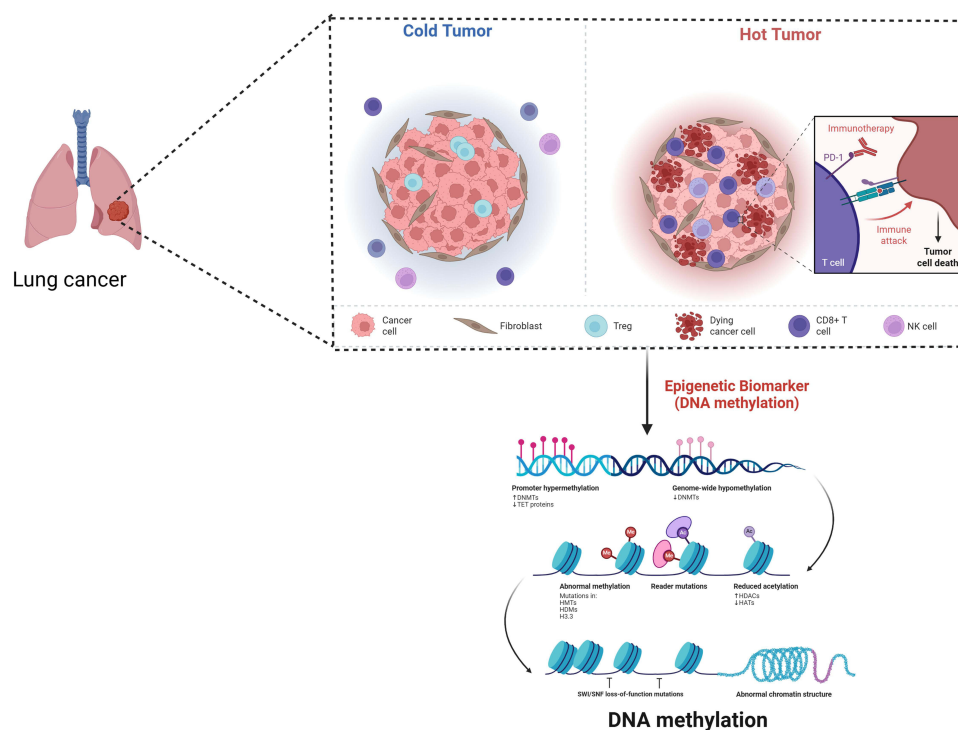


Figure 1 The role of DNAm biomarkers in enhancing immunotherapy and noninvasive diagnosis of lung cancer. Schematic overview of how DNA methylation (DNAm) shape “cold” versus “hot” tumor immune phenotypes in lung cancer, influencing immune cell infiltration, PD-1/PD-L1 expression, and response to immunotherapy (Created in BioRender).

In cancer, aberrant methylation, such as promoter hypermethylation of tumor suppressor genes, can disrupt normal gene regulation, activate oncogenes, and contribute to tumor progression. In lung cancer, alterations in DNAm affect key genes, including CDKN2A, MLH1, TERT, EGFR, KRAS, BRAF, and HER2, thereby influencing tumor growth, metastasis, and therapeutic response.^{21,22} Environmental factors exacerbate epigenetic changes. Promoter hypermethylation can silence tumor suppressor and DNA repair genes, whereas global hypomethylation may lead to chromosomal instability and increased mutation rates, further driving tumorigenesis.⁶ DNAm also affects the tumor immune micro-environment and response to immunotherapy. Silencing of DNA mismatch repair genes, such as MLH1, can induce MSI and elevate TMB, generating neoantigens²³ that enhance tumor immunogenicity and sensitivity to ICIs.^{16,17} Aberrant methylation can modulate immune regulated genes and antigen presentation, influencing T cell infiltration and activity.^{24,25} Beyond its predictive value, DNAm is a promising minimally invasive biomarker for lung cancer detection. Analysis of tumor specific methylation patterns in bronchial aspirates, sputum, or circulating cell-free DNA facilitates early diagnosis, risk assessment, and monitoring of therapeutic response without the need for invasive biopsies.²¹ Integrating methylation data with other biomarkers, such as PD-L1 expression, TMB, and TILs, can further refine patient selection and optimize immunotherapy outcomes. In summary, DNAm is a critical epigenetic mechanism that links tumor biology, immune regulation, and therapeutic responses in lung cancer. Its dysregulation contributes to tumor initiation, progression, and immune evasion, and provides measurable biomarkers for early detection, prognosis, and immunotherapy prediction. Continued research on methylation profiling and its integration with other biomarkers holds substantial potential for enhancing precision medicine approaches in lung cancer management.

Epigenetic Alterations and Immunotherapeutic Implications in Lung Cancer

Compromised immunity significantly contributes to the development of lung cancer, whereas maintaining immune equilibrium is crucial for overall well-being. Specific genes like EGFR, HER2, BRAF, KRAS, CDKN2A, TERT, etc., play active roles in regulating immune balance in lung cancer, with distinct DNAm patterns observed in smoking-associated case.^{21,22} A study conducted by Lin et al (2009) reported notable gene alterations in patients with stage I NSCLC, genes such as RASSF1A (42.74%), APC (39.52%), ESR1 (29.84%), ABCB1 (24.19%), MT1G (20.16%), and HOXC9 (13.71%) exhibited higher methylation frequencies compared to non-cancerous lung lesions. The p16INK4A gene showed methylation in 22.56% of cancerous tissues and 7.69% of non-cancerous tissues.²⁶

Another study focused on genes downregulated due to chronic cigarette-smoke-extract (CSE) exposure in human airway epithelial cells (HAEC) and their hypermethylation in lung adenocarcinoma (LUAD). Among these genes, CCNA1, SNCA, and ZNF549 had reduced expression in COPD lung tissues compared to non-COPD cases. CCNA1 and SNCA were further suppressed in tumors with COPD. The genes promoters were hypermethylated in LUAD but not in normal or COPD lungs. Validation using Cancer Genome Atlas data confirmed their altered expression and hypermethylation in LUAD. Importantly, SNCA and ZNF549 methylation in sputum DNA from LUAD cases (52% and 38%) surpassed cancer-free smokers (26% and 15%) ($P < 0.02$).²⁷ Notably, methylation of SNCA and ZNF549 detected in sputum DNA from LUAD cases was more prevalent compared to cancer-free smokers. This indicates that genes highly expressed in cancer undergo methylation and are presented in Table 1. It summarizes the DNAm patterns observed in key genes associated with lung cancer. Tumor suppressor genes, such as RASSF1A, APC, P16, DAPK, and CDKN2A, frequently exhibit hypermethylation, whereas oncogenes, including EGFR and TERT, are often hypomethylated, facilitating uncontrolled cellular proliferation and immortality. Additionally, transcriptional regulators and genes involved in lung development, such as GATA4, PAX5 α , ESR1, and HOXC9, also demonstrate hypermethylation, whereas genes such as HER2, BRAF, and KRAS are seldom methylated. Environmental factors, notably smoking, further modulate these methylation patterns, as evidenced by the hypermethylation of SNCA and ZNF549 in LUAD, which is detectable in-patient sputum. Collectively, these findings suggest that aberrant DNAm is prevalent in lung cancer and reflects functional alterations pertinent to tumorigenesis, immune regulation and disease progression.

The identification of methylation signatures holds significant potential for early, noninvasive risk assessment in high-risk populations. When combined with immunotherapy strategies, these biomarkers may enhance the precision of treatment selection. For instance, in patients exhibiting PD-L1 expression levels of $\geq 50\%$, immunotherapy has been shown to significantly enhance 5-year survival rates (32.9%) compared to chemotherapy (16.3%).⁴⁶ However, only

Table 1 Epigenetic Alterations and Immunotherapeutic Implications in Lung Cancer

Sl.No.	Gene	Description	Function	DNA Methylation Status	Immunotherapy Implication	PMID
1.	EGFR	Epidermal growth factor receptor	Cell growth, survival, proliferation	Hypomethylated (often)	EGFR pathway activation is associated with immune-cold phenotypes and reduced ICI efficacy; methylation changes may affect sensitivity to targeted therapy and immune responses.	34447695 ²⁸
2.	E-cadherin	Epithelial Cadherin	Cell adhesion, maintenance of epithelial integrity	Hypermethylated	E-cadherin hypermethylation linked to better survival; may indicate improved immunotherapy response.	24906605 ²⁹
3.	RAR β 2	Retinoic Acid Receptor Beta 2	Cellular differentiation, proliferation, apoptosis, and embryonic development	Hypermethylated	RAR β 2 hypermethylation may indicate reduced immunotherapy response in never-smoker lung adenocarcinoma patients.	25806093 ³⁰
4.	PI6	Cyclin-Dependent Kinase Inhibitor 2A	Tumor suppressor, cell cycle regulation	Hypermethylated	Loss of cell-cycle control can increase tumor aggressiveness and genomic instability; may indirectly influence neoantigen landscape and ICI outcomes.	36153611 ³¹
5.	MGMT	O-6-Methylguanine-DNA Methyltransferase	DNA repair, alkyl adduct removal	Hypermethylated	MGMT silencing may increase sensitivity to alkylating agents and influence mutational patterns that alter immunogenicity; possible impact on combination regimens.	37200021 ³²
6.	GATA4	GATA Binding Protein 4	Transcription factor, lung development	Hypermethylated	Altered differentiation and the tumor microenvironment, potentially affecting immune surveillance and response to ICIs.	36153611, 38843657 ^{31,33}
7.	PAX5 α	Paired Box 5 α	Transcription factor, B-cell development	Hypermethylated	Reduced PAX5 signaling may affect humoral immune components and antigen presentation pathways; possible indirect effects on ICI responses.	36153611, 17658281 ^{31,34}
8.	DAPK	Death-Associated Protein Kinase I	Apoptosis induction, tumor suppressor	Hypermethylated	DAPK1 loss limits immunogenic cell death, potentially reducing neoantigen release and diminishing ICI efficacy.	36153611, 38142898 ^{31,35}
9.	ABCB1	ATP-Binding Cassette Sub-Family B Member 1	Efflux pump transporter, drug resistance	Hypermethylated (Promoter)	Epigenetic regulation of ABCB1 may influence chemo-immunotherapy combinations and drug exposure that modulate immune responses.	19506903, 24332606 ^{26,36}

(Continued)

Table 1 (Continued).

Sl.No.	Gene	Description	Function	DNA Methylation Status	Immunotherapy Implication	PMID
11.	APC	Adenomatous Polyposis Coli	Wnt pathway regulator; tumor suppressor	Hypermethylated (Promoter)	Wnt pathway dysregulation is linked to T-cell exclusion; APC silencing may contribute to immune-cold phenotype and resistance to ICIs.	18841156, 31546933 ^{37,38}
12.	RASSF1A	Ras Association Domain Family Member 1A	Tumor suppressor; apoptosis, cell cycle	Hypermethylated (Promoter)	RASSF1A loss is associated with genomic instability and immune evasion mechanisms; may predict poorer ICI outcomes or require combined epigenetic therapy.	19506903, 31546933, 23806794 ^{26,38,39}
13.	ESR1	Estrogen Receptor 1	Hormone receptor; transcription regulation	Hypermethylated (Promoter)	Hormone-signaling changes may modulate immune pathways; lung-specific effects on ICI response are uncertain and not established as a robust biomarker.	19506903, 36766706 ^{26,40}
14.	HOXC9	Homeobox C9	Transcription factor; development	Hypermethylated (Promoter)	HOX gene silencing can alter differentiation and antigenic programs, potentially affecting immune recognition and prognostic stratification.	17369352, 19506903 ^{20,26}
15.	HER2	Human epidermal growth factor receptor	Cell growth, survival, differentiation	Not commonly hypermethylated; activation typically via amplification/overexpression	HER2 alterations can shape immune contexture and influence responsiveness to targeted therapies and combinations with ICIs.	18757082 ⁴¹
16.	BRAF	B-Raf Proto-Oncogene	Cell growth, proliferation, signaling	Hypermethylated (rarely)	BRAF pathway changes influence tumor immunogenicity; epigenetic silencing is uncommon but may modulate immune signaling when present.	29235923 ⁸
17.	KRAS	Kirsten rat sarcoma virus	Cell growth, signaling	Hypermethylated (rarely)	KRAS-mutant tumors (especially with smoking signature) often show higher TMB and may respond differently to ICIs;	32576853 ⁴²
18.	CDKN2A	Cyclin-Dependent Kinase Inhibitor 2A	Tumor suppressor; regulates cell cycle	Hypermethylated (Promoter)	CDKN2A inactivation can increase proliferation and genomic instability; may affect neoantigen generation and ICI sensitivity.	34625620 ⁴³
19.	TERT	Telomerase Reverse Transcriptase	Maintains telomere length, cellular immortality	Hypomethylated (Promoter)	TERT activation supports immortality and can modulate immune escape mechanisms; methylation changes may reflect aggressive biology relevant to immunotherapy outcomes.	38278800 ⁴⁴

(Continued)

Table 1 (Continued).

Sl.No.	Gene	Description	Function	DNA Methylation Status	Immunotherapy Implication	PMID
20.	SOCS1	Suppressor of Cytokine Signaling 1	Negative regulator of cytokine signaling	Hypermethylated (Promoter)	SOCS1 loss deregulates cytokine signaling and may create pro-tumor inflammatory states; effects on ICI response are complex and context-dependent.	14617776, 41019358 ^{14,45}

a subset of patients (46%) experienced sustained benefits, and the incidence of immune-related adverse events remains a concern (22%). For individuals with PD-L1 expression <1%, the combination of immunotherapy and chemotherapy has been observed to more than double response rates, increasing from 14.3% to 32%.⁴⁶ These findings highlight the limitations of relying solely on PD-L1 expression for treatment stratification in NSCLC. Although a high tumor mutational burden (TMB) is associated with improved immunotherapy outcomes, it is currently inadequate for precise clinical decision making.⁴⁷ Consequently, there is a need for more robust and biologically informative biomarkers to predict therapeutic response, resistance, and immune-related adverse events.

Efficacy Biomarkers for Immunotherapy in Lung Cancer

Biomarkers are integral for elucidating tumor biology, forecasting disease progression, and informing therapeutic strategies for lung cancer. They offer quantifiable indicators of genomic alterations, immune activity, and microenvironmental conditions that collectively affect clinical outcomes. Given the genetic and immunologic heterogeneity of lung cancer, the identification of reliable biomarkers is crucial for evaluating prognosis and determining the potential efficacy of novel immunotherapeutic interventions. These biomarkers can be broadly categorized into genomic and non-genomic biomarkers. Genomic biomarkers include inherited or acquired alterations in the tumor genome, such as mutations, copy number variations, and deficiencies in DNA repair pathways. Metrics such as TMB⁴⁸ and MSI,⁴⁹ reflect the extent of genomic instability and can indirectly suggest the presence of neoantigens capable of eliciting antitumor immune responses.⁵⁰ Genomic alterations in immune regulatory genes further affect antigen presentation, interferon signaling, and immune evasion mechanisms within the tumor microenvironment. In contrast, non-genomic biomarkers capture the dynamic aspects of tumor-immune interactions. These include PD-L1 protein expression, which represents a mechanism by which tumor and immune cells modulate T cell activity, TILs, which indicate ongoing immune engagement,⁵¹ and immunosuppressive cytokines, which delineate the degree of immune inhibition or activation within the tumor microenvironment in biological samples.⁵² These biomarkers provide functional insights into the immune landscape that genomic alterations alone cannot fully elucidate.

In summary, biomarkers provide valuable insights into biological processes and can be broadly divided into genomic markers (genetic traits) and non-genomic markers (various biological factors), all of which are pivotal in disease assessment, prognosis, and treatment response evaluation.

PD-L1

PD-L1 (programmed death-ligand 1) is a transmembrane protein belonging to the B7 family that is expressed on tumor cells as well as various immune and non-immune cells. It interacts with PD-1 on activated T cells, delivering an inhibitory signal that suppresses T cell activation and cytotoxic activity, thereby facilitating immune evasion. Pembrolizumab, an anti-PD-1 antibody, targets this interaction to restore antitumor immunity in NSCLC.⁵³ Initial studies have indicated that PD-L1 protein levels may predict response to pembrolizumab; however, the predictive value is complicated by PD-L1 expression on both tumor and immune cells.⁵⁴ In a study (registration number JapicCTI-184038), involving 39 patients with stage IV NSCLC, 64.1% exhibited partial responses, with 12-month progression-free survival

(PFS) and overall survival rates of 54.9% and 70.6%, respectively.⁵⁵ In the ongoing KEYNOTE-189 trial (NCT02578680), pembrolizumab combined with chemotherapy demonstrated improved outcomes and patient-reported quality of life compared to chemotherapy alone.⁵⁶

Recent evidence also indicates that epigenetic mechanisms regulate PD-L1 transcription and may influence immunotherapy responses. DNAm at CpG sites in the PD-L1 promoter is inversely correlated with mRNA expression in NSCLC cell lines, and experimental hypermethylation reduces PD-L1 levels, although the clinical correlations are modest.⁵⁷ Genome-wide methylation profiling of patients with NSCLC receiving anti-PD1 therapy revealed differentially methylated promoters and enhancers associated with response and survival, suggesting that the epigenetic context may modulate therapeutic efficacy.¹⁰ Histone modifications and non-coding RNA also contribute to PD-L1 regulation, underscoring the multi-layered control of immune checkpoint expression.⁵⁸ These findings suggest that PD-L1 are also regulated by epigenetic mechanisms, which contribute to its variability across patients. These multilayered regulatory factors highlight the necessity of combining PD-L1 protein assessment with epigenetic markers to enhance clinical utility.

Tumor Mutational Burden (TMB)

TMB is the count of somatic mutations within a specific portion of a tumor genome, typically expressed as mutations per megabase (mut/Mb).⁵⁹ Certain non-synonymous somatic mutations can alter proteins in a way that the immune system might perceive them as foreign. These modifications can lead to the creation of neoantigens, unique tumor-specific peptides presented by MHC molecules and recognized by T cells, thus triggering an antitumor immune response. TMB, which indirectly indicates the number of neoantigens, is used as a predictive biomarker for Immunotherapy (ICI) treatment. A high TMB suggests a higher likelihood of having immunogenic neoantigens.¹² TMB varies across tumor types and in patients. In tumors with elevated TMB, such as melanoma and lung cancers, there is emerging evidence linking TMB to the presence of neoantigens. Neoantigens can stimulate T-cell reactivity, leading to an immune response against tumors. ICIs, such as anti-PD-1/PD-L1 and anti-CTLA-4 enhance T-cell activity against tumors. Therefore, TMB or neoantigen load could guide treatment decisions for ICIs.⁶⁰ Although not all mutations create immunogenic neoantigens, TMB quantifies mutations in a tumor and informs treatment choices. Recent clinical data suggest that tumors with a high TMB are more likely to respond well to ICIs.⁶¹ Studies have primarily focused on NSCLC but have also extended to melanoma, head and neck squamous cell carcinoma, small cell lung cancer, and urothelial carcinoma.⁶⁰ High TMB has been linked to better outcomes with anti-PD-1/PD-L1 and anti-CTLA-4 therapy. Notably, studies in NSCLC patients treated with certain inhibitors have confirmed TMB's predictive value of TMB for PFS. The increasing number of studies on TMB as a predictor of ICIs response demonstrates the growing interest in this area.⁵¹ While one of the studies shows TMB has displayed potential in retrospectively predicting the benefits of PD-L1/PD-1 inhibitors. To prospectively evaluate blood TMB (bTMB), a Phase 2 trial named B-FIRST (NCT02848651) was conducted. This study involved 152 participants with locally advanced or metastatic stage IIIB–IVB NSCLC. The primary objectives were to compare objective response rate (ORR) and PFS between high and low bTMB subgroups, with a predefined bTMB cutoff of ≥ 16 (equivalent to 14.5 mut/Mb). While investigator assessed PFS between the bTMB ≥ 16 and bTMB < 16 groups did not show statistical significance, a higher ORR was observed with bTMB ≥ 16 . Additionally, increasing bTMB cutoffs correlated with improved ORR. No new safety concerns arose. Exploratory analysis revealed that patients with maximum somatic allele frequency (MSAF) below 1% exhibited greater ORR compared to those with MSAF at or above 1%. However, this effect seemed to stem from better baseline prognostic factors rather than MSAF itself. In a subsequent exploratory analysis at the 36.5-month follow-up, bTMB ≥ 16 was associated with longer overall survival (OS) than bTMB < 16 . Further research and refinement of the assay are needed to establish bTMB as a predictive, standalone biomarker for immunotherapy or for combined use with other biomarkers.¹¹

While another study shows, between January 15, 2016, and June 25, 2019, a total of 1073 patients were enrolled in the study. By June 27, 2019, a total of 1066 participants were treated, with 805 (76%) evaluable for TMB assessment and 105 (13%) of these had high TMB (tTMB-high) status. Among the patients enrolled at least 26 weeks before the data cutoff, 790 (75%) were included in efficacy analyses. Of these, 102 (13%) had tTMB-high status (≥ 10 mut/Mb), while 688 (87%) had non-tTMB-high status (< 10 mut/Mb). The median study follow-up was 37.1 months. Results showed that

the tTMB-high group had an objective response rate of 29%, compared to 6% in the non-tTMB-high group. Among 105 patients, 11 (10%) experienced serious adverse events related to treatment, and 16 (15%) had severe adverse events. Colitis was the primary severe adverse event, occurring in two patients. One patient's death due to pneumonia was considered treatment-related by the investigator.⁶²

It is a valuable biomarker for predicting responses to immunotherapy, particularly in certain cancer types like NSCLC and melanoma. It quantifies the mutational load in a tumor and indirectly reflects the presence of immunogenic neoantigens. High TMB is associated with better outcomes and higher response rates to ICIs. However, TMB's applicability can vary among tumor types, not all mutations are immunogenic, and refinement of TMB assays is needed. Despite these challenges, TMB holds promise as a predictive biomarker for immunotherapy, however, ongoing research is required to fully establish its clinical utility.

Beyond genetic mutations, recent epigenetic studies have highlighted an additional layer of regulation influencing TMB. Recent research has demonstrated that epigenetic dysregulation plays a significant role in increasing the TMB by fostering genomic instability and compromising the DNA repair pathways. For instance, hypermethylation of the MLH1 promoter, a well-documented epigenetic mechanism, results in mismatch repair deficiency, thereby substantially elevating the mutational load across various cancers, including NSCLC.⁶³ Another study of NSCLC further revealed that global DNA hypomethylation and loss of methylation at DNA repair gene promoters are associated with a higher TMB and enhanced responses to PD-1/PD-L1 inhibitors.⁶⁴ Integrative methylation mutation profiling has shown that epigenetic silencing of homologous recombination (HR) repair genes correlated with an increased mutational burden and significant benefits from immunotherapy. These findings suggest that incorporating epigenetic markers of DNA repair dysfunction alongside TMB could improve the prediction of ICIs responses in NSCLC.⁶⁵ Collectively, these findings highlight that integrating TMB with epigenetic markers of DNA repair dysfunction may substantially improve the ability to predict patient responses to ICIs therapy in NSCLC.

Microsatellite Instability (MSI)

MSI is characterized by variable lengths of microsatellite sequences in the genome and is present in conditions such as Lynch syndrome and sporadic cancers, such as lung, gastric, and endometrial cancers. In sporadic cases, abnormalities in DNA MMR due to gene mutations or methylation of the MLH1 gene can lead to gene mutation accumulation, fueling cancer progression.⁶⁶ DNA repair deficiencies can result in elevated TMB, which is detected through targeted sequencing and indicates dMMR arising from inherited or somatic MMR gene mutations.⁶⁷ This instigates MSI and mutational accumulation, generating immunogenic neoantigens and a high TMB. High MSI tumors are associated with heightened PD-L1 expression and robust anti-PD-1 responses.⁶⁸ In a study involving 44 patients with diverse cancers, treatment with TAS-116 demonstrated safety, established recommended dosage, and yielded a 16% objective tumor response across various cancer types, even in microsatellite stable cancers.⁶⁹ Another study with 120 participants, 42% of whom were PD-L1 positive, displayed varying objective response rates to atezolizumab monotherapy across different cancers. Notably, PD-L1 positive patients exhibited significantly improved responses with combination therapy.⁷⁰ In a separate investigation (NCT02908906), a Phase 1 trial involving 58 patients treated with cetrelimab unveiled two recommended phase 2 doses: 240 mg every two weeks or 480 mg every four weeks. Following the initial dose, serum concentrations ranged from 24.7 to 227.0 µg/mL, with a median time to maximum concentration between 2.0 to 3.2 hours. Consistent pharmacodynamic effects were sustained across doses during the dosing period. Progressing to phase 2, 146 patients received the 240 mg Q2W dose, with 53.9% encountering grade ≥ 3 adverse events. Immune-related adverse events were observed in 35.3% of patients, with 6.9% being of grade ≥ 3 severity. Overall response rates varied among different tumor types, with notable rates observed in NSCLC (34.3%), PD-L1-high NSCLC (52.6%), melanoma (28.0%), and MSI-H CRC (23.8%).⁷¹

It is a promising biomarker for immunotherapy, with both advantages and limitations. Its strengths lie in its ability to predict immunotherapy responses effectively, particularly in cancers marked by DNA mismatch repair deficiencies, resulting in elevated TMB and the generation of immunogenic neoantigens. However, MSI's applicability is restricted to specific cancer types with MMR deficiencies, limiting its universal utility. Integrating MSI with complementary

biomarkers such as TMB and PD-L1 can improve patient stratification. Overall, combining these markers may optimize immunotherapy outcomes and guide clinical decision-making.

Tumor-Infiltrating Lymphocytes (TILs)

TILs have shown potential as predictive biomarkers but require further exploration and validation before they can be used in clinical settings. TILs, consisting of different fractions of CD4+ (helper) and CD8+ (cytotoxic) T lymphocytes, constitute a major component of the tumor microenvironment (TME).⁷² Infiltrated immune cells in tumors, including T cells, B cells, myeloid lineage leukocytes, natural killer (NK) cells, macrophages, and dendritic cells, contribute to either pro- or anti-tumor activities. Among these infiltrating cells, TILs (T cells, B cells, and NK cells) are crucial determinants of the host immune response against tumor cells, responsible for initiating antitumor immune responses and potentially targeting tumor antigens for cell killing.⁷³

In one study, high PD-L1 expression (>40 with a combined positive score) was significantly more prevalent in patients who achieved disease control. Additionally, a high TMB (>10 mut/Mb) was associated with improved OS and PFS. High PD-L1 expression was associated with longer OS and PFS. However, high tumor-infiltrating lymphocyte counts (>1.2) did not significantly impact OS or PFS. Patients with both high TMB and PD-L1 expression had notably better OS and PFS outcomes.⁷⁴

Another study involving resection tumors from nine cancer types and biopsies from NSCLC patients enrolled in a phase 1/2 clinical trial investigating PD-L1 antibody therapy (durvalumab, NCT01693562), immunostaining was conducted to assess CD8+ T cell densities. Whole-slide scanning and image analysis using customized algorithms were performed, with validation against pathologist scoring across various CD8+ TIL densities in different cancers. This analysis confirmed the reliability of CD8+ T cell density as a biomarker, providing valuable insights into immunotherapy responses in diverse cancer types.⁷⁵

TILs are promising predictive biomarkers owing to their role in shaping the immune response against tumors, including detection and targeting tumor cells. High PD-L1 expression and TMB are associated with improved patient outcomes in terms of OS and PFS. However, TIL populations can be heterogeneous, limiting their predictive value in various cancers and patient groups. However, heterogeneity in TIL populations and the complexity of assessment limit their standalone predictive value, highlighting the need for integration with other biomarkers such as PD-L1, TMB or DNAm to optimize immunotherapy stratification.

Immunosuppressive Cytokine

Immunosuppressive cytokines play a critical role in establishing an immune-suppressed environment in lung cancer, impeding the body's capacity to mount an effective antitumor immune response. The key immunosuppressive cytokines in lung cancer include TGF- β , IL-10, IL-6, IL-4, IL-13, VEGF, and PGE2. These cytokines can inhibit immune cell function, promote immune cell polarization toward immunosuppressive phenotypes, and hinder the immune system's ability to recognize and attack cancer cells.^{76,77}

Researchers are actively exploring strategies to target these immunosuppressive cytokines and their pathways in immunotherapy development to restore the body's immune response against lung cancer. While these cytokines can prevent excessive immune responses, targeting them may lead to therapy resistance and side effects, underscoring the need to comprehensively understand and address their role in lung cancer for more effective treatments.

In a clinical study conducted between October 2015 and August 2020, Phase I revealed the absence of dose-limiting toxicities. In Phase II, 25 NSCLC patients received galunisertib and nivolumab, with common treatment-related adverse events including pruritus, fatigue, and decreased appetite. Notably, no severe grade 4 or 5 treatment-related adverse events were observed. Several patients achieved partial responses, stable disease, and one even showed a partial response after an initial pseudo-progression. Interferon gamma response genes increased post-treatment, while cell adhesion genes were repressed, although limited sample size hindered the clinical significance assessment.⁷⁸

In another Phase 3 trial, researchers investigated combining erlotinib and bevacizumab in 311 untreated advanced NSCLC patients. The combination group exhibited significantly improved PFS compared to the erlotinib-only group,

extending even to patients with brain metastases. However, the combination therapy group reported a higher occurrence of grade ≥ 3 treatment-related adverse events.⁷⁹

Additionally, a separate study evaluated the effects of *G. lucidum* treatment on immune parameters. The *G. lucidum* group showed a higher prevalence of certain immune cell types, such as CD3 + CD4 +, and lower prevalence of immunosuppressive cell types like CD4 + CD25 + Treg and CD3 + HLADR + cells compared to the control group. IL-12 levels increased during treatment, negatively impacting IL-10 levels. Other immunosuppressive factors like COX2 and TGF- β 1 were less prevalent in treated patients. Correlation analysis revealed associations between various immune markers, and Kaplan-Meier analysis suggested certain markers may predict treatment benefits related to PFS and OS.⁸⁰

Targeting immunosuppressive cytokines like TGF- β , IL-10, and IL-6 in lung cancer offers promise for immunotherapy but also presents challenges. These cytokines create an immune-suppressed environment within tumors, hindering effective anti-tumor immune responses. Some combination therapies show acceptable safety profiles and improved PFS, even in patients with brain metastases. However, there is a risk of therapy resistance, increased severe treatment-related adverse events, and limitations in assessing clinical significance due to sample size constraints.^{78–80}

Integrated Epigenetic Signatures

Epigenetic biomarkers, involving DNAm, have emerged as a promising avenue for improving cancer diagnosis.⁸¹ They offer distinct advantages over other markers like gene expression or genetic signatures. DNAm changes are stable and occur early in cancer development.⁷ Furthermore, DNAm can be detected using sensitive and cost-effective techniques, even in samples with low tumor content. Notably, this epigenetic modification can also be identified in various bodily fluids, making it a potential tool for noninvasive and minimally invasive cancer detection.⁸²

Despite their promise, the clinical implementation of epigenetic biomarkers has been limited, primarily because of the lack of large-scale validation studies and challenges in standardizing analytical methods.⁸² Additionally, many studies have focused on individual genes driven by specific hypotheses, although more comprehensive genome-wide approaches are now emerging. High-throughput epigenomic studies, which allow for unbiased data-driven research, have the potential to uncover novel and robust cancer biomarkers.⁸³

Currently, bronchoscopic examination and cytological specimen analysis are the most common methods for lung cancer diagnosis. However, nearly half of the cases, especially those with peripheral tumors, remain undetected. This often requires invasive procedures, such as surgical lung biopsies or transthoracic needle biopsies, which carry significant risks.⁸⁴ Incorporating molecular biomarkers, including epigenetic changes and gene expression patterns, into bronchial aspirates or sputum samples offers a promising avenue for enhancing the accuracy of noninvasive and minimally invasive cancer diagnosis. These biomarkers can also be used to develop predictive tools, such as nomograms, enabling personalized risk assessment and advancing personalized medicine.

Epigenetic abnormalities are common in cancer and contribute to cancer initiation, progression, and treatment response. In NSCLC, hypermethylation of CpG-rich sequences in gene promoters, known as CpG islands, is associated with various factors such as smoking, histological subtype, disease progression, molecular subtypes, and patient prognosis. Through a comprehensive analysis of DNAm changes in three cohorts, HOXA9 promoter methylation has emerged as a potential prognostic biomarker. The prognostic value of HOXA9 promoter methylation was further evaluated using pyrosequencing in 217 primary tumors from two cohorts, both individually and in combination with mRNA and miRNA. Statistical analyses were conducted separately for each cohort and then combined. Whether used alone or in conjunction with mRNA and miRNA biomarkers, HOXA9 promoter methylation provides valuable prognostic information for patients with stage I lung adenocarcinoma.⁸⁵

Recent studies have identified additional DNAm markers with significant diagnostic and prognostic potential. Du et al (2024) reported a seven-gene methylation panel (TAC1, CDO1, HOXA9, ZFP42, SOX17, RASSF1A, SHOX2) that effectively detects early-stage lung cancer using blood cell-free DNA (cfDNA). In a study involving 149 lung cancer patients, five benign cases, and 48 healthy controls, a lasso-logistic regression model incorporating age, sex, and methylation Δ Ct values achieved 86.7% sensitivity, 81.4% specificity, and an area under the curve (AUC) of 0.891. The panel also distinguished ground-glass nodules (GGNs) from mass-type tumors (AUC 0.753), with CDO1 and SHOX2 methylation levels varying across cancer stages. This seven-gene panel demonstrated a strong potential

for noninvasive lung cancer diagnosis and tumor characterization.⁸⁶ Huang et al (2024) demonstrated that multiple DNAm regulators possess significant prognostic and immunogenic relevance in lung adenocarcinoma.³ Yang et al (2024) demonstrated that integrating cfDNA methylation with protein markers and low-dose computed tomography (LDCT) imaging further improved the differentiation of pulmonary nodules, achieving an AUC of 0.925 overall and 0.951 for small nodules (5–10 mm). Collectively, these findings underscore the efficacy of combining molecular and imaging features for accurate and noninvasive lung cancer diagnosis and tumor characterization.⁸⁷ A systematic review by Dolcini et al (2025) highlighted consistently validated DNA biomarkers for lung cancer. Their pooled analysis indicated that higher blood DNAm levels were significantly associated with increased LC risk (odds ratio [OR] 1.24, 95% confidence interval [CI] 1.10–1.39), with a stronger association in prospective cohort studies (OR 1.61) than in case–control studies (OR 1.05). Sensitivity analyses confirmed the robustness of these findings, suggesting that elevated blood DNAm levels may serve as a promising noninvasive biomarker for lung cancer risk assessment, in long-term population studies.⁸⁸ Additionally, Li et al (2025) reported that distinct DNAm signatures can predict recurrence risk in stage I NSCLC cases, those harboring EGFR mutations.⁸⁹ Several ongoing clinical trials have highlighted the translational potential of DNAm and epigenetic biomarkers (Table 2). These studies explored early detection through noninvasive sampling methods, the prognostic and immunogenic relevance of DNAm, and their integration with immunotherapy and epigenetic drug combinations, such as hypomethylating agents (azacitidine, decitabine, and guadecitabine) and immune checkpoint inhibitors (nivolumab, pembrolizumab, and durvalumab). Additionally, some trials have aimed to develop and validate epigenetic assays for diagnosis, treatment monitoring, and personalized risk stratification. Collectively, these findings underscore the clinical relevance of DNAm biomarkers as robust tools for early detection, prognosis, and therapy guidance, supporting their integration into modern personalized approaches for lung cancer management.

Future Prospects and Challenges

Future prospects in the field of epigenetic biomarkers for immunotherapy in lung cancer are promising but come with share of challenges. First, epigenetic biomarker discovery efforts should be expanded. While DNAm has shown potential,

Table 2 Ongoing Clinical Trials Testing Epigenetics Immunotherapy in Patients Affected by Lung Cancer (NSCLC & SCLC; Data Obtained From <https://clinicaltrials.gov/>)

Sl. No.	NCT Number	Study Title	Conditions	Interventions	Sex	Study Phases	Start Date
1.	NCT02511288	Liquid Biopsies in Patients Presenting Non-small Cell Lung Cancer	Carcinoma, Non-Small-Cell Lung Cancer	NA	All	NA	2015-07
2.	NCT02250326	Safety and Efficacy Study of Nab [®] -Paclitaxel With CC-486 or Nab [®] -Paclitaxel With Durvalumab, and Nab [®] -Paclitaxel Monotherapy as Second/Third-line Treatment for Advanced Non-small Cell Lung Cancer	Carcinoma, Non-Small-Cell Lung Cancer	DRUG: nab-paclitaxel IV DRUG:CC 486 DRUG: Durvalumab	All	II	2015-01
3.	NCT04965129	Supplementation of n-3 PUFA in the Modulation of Lean Mass in Patients With Lung Cancer Receiving a High-protein Diet	Lung Cancer	DRUG: Placebo DRUG: Fish oil	All	NA	2022-11

(Continued)

Table 2 (Continued).

SI. No.	NCT Number	Study Title	Conditions	Interventions	Sex	Study Phases	Start Date
4.	NCT03220477	Pembrolizumab (Immunotherapy Drug) in Combination With Guadecitabine and Mocetinostat (Epigenetic Drugs) for Patients With Advanced Lung Cancer.	Lung Cancer	DRUG: Pembrolizumab DRUG: Guadecitabine DRUG: Mocetinostat	All	I	2017-08
5.	NCT04926181	Apalutamide Plus Cetrelimab in Patients With Treatment-Emergent Small Cell Neuroendocrine Prostate Cancer	Small Cell Neuroendocrine Carcinoma Prostate Cancer Small Cell Carcinoma	DRUG: Apalutamide BIOLOGICAL: Cetrelimab	Male	II	2022-03
6.	NCT02664181	Rational Epigenetic Immunotherapy for Second Line Therapy in Patients With NSCLC: PRECISE Trial	Lung Cancer Non-small Cell Lung Cancer	DRUG: Nivolumab DRUG: oral decitabine DRUG: Tetrahydrouridine	All	II	2017-06
7.	NCT06717243	Genomic and Methylation Markers in SCLC and LCNEC for Chemo-Immunotherapy Resistance Prediction (STRATUS)	Extensive-stage small cell lung cancer (ES-SCLC) locally advanced or metastatic large cell neuroendocrine carcinoma (LCNEC)	Platinum-based chemotherapy (cisplatin or carboplatin) combined with immune checkpoint inhibitors (atezolizumab or durvalumab)	All	NA	2025-02
8.	NCT05707585	Lung Cancer Diagnosis by Detecting Epigenetic Imprinting Alterations in Bronchoalveolar Lavage	Lung cancer	NA	All	NA	2023-01
9.	NCT07008664	Signature Development and Validation Protocol for an Epigenetic Assay in Diagnosing Lung Cancer	Lung cancer	Epigenetic Signature Assay	All	NA	2025-03
10.	NCT00242723	Evaluation of Cell Changes in Blood and Tissue in Cancers of the Lung, Esophagus and Lung Lining	Lung cancers or esophageal cancers, malignant pleural mesotheliomas, mediastinal or chest wall neoplasms, thymoma/ thymic carcinomas, or thoracic metastases from cancers of non-thoracic origin	Endoscopic biopsies of tumor and adjacent normal tissues, and to provide blood and urine samples	All	NA	2005-11
11.	NCT04814407	Developing Novel Circulating Epigenetic Biomarkers for Early Detection of Lung Cancer	Lung cancer	Blood-derived DNA methylation detection	All	NA	2021-03
12.	NCT01139944	Biomarkers in Tissue and Blood Samples From Patients With Early-Stage Non-Small Cell Lung Cancer	Lung cancer	DNA methylation analysis/polymerase chain reaction/ laboratory biomarker analysis	All	NA	2010-07

(Continued)

Table 2 (Continued).

Sl. No.	NCT Number	Study Title	Conditions	Interventions	Sex	Study Phases	Start Date
13.	NCT06838806	Epigenetic Nucleosomes in Plasma for Pulmonary Nodule Differentiation	Lung Nodule	Blood draw	All	NA	2025-03
14.	NCT06963307	Novel Epigenetic Mapping in Cell-Free DNA for the Detection of Lung Cancer (LumenDx)	Lung Cancer, Non-Small Cell Lung cancer	Blood will be taken before surgery / treatment and after surgery / treatment at the indicated time points	All	NA	2025-05
15.	NCT01928576	Phase II Anti-PD1 Epigenetic Therapy Study in NSCLC.	Non-Small Cell Lung Cancer	Drug: Azacitidine Drug: Entinostat Drug: Nivolumab Drug: CC-486 300	All	II	2013-11
16.	NCT02146170	Tissue Procurement and Natural History Study of People With Non-Small Cell Lung Cancer, Small Cell Lung Cancer, Extrapulmonary Small Cell Cancer, Pulmonary Neuroendocrine Tumors, and Thymic Epithelial Tumors	Non-Small Cell Lung Cancer /Small Cell Lung Cancer/ Extrapulmonary Small Cell Cancer/ Pulmonary Neuroendocrine Tumors/ Thymic Epithelial Tumors	Patients with histologically or cytologically confirmed NSCLC, SCLC, ESCC, PNET, and TET	All	NA	2014-05
17.	NCT05102110	Feasibility Study to Investigate Rectal Mucus in Aero-Digestive Tract Cancer	Non Small Cell Lung Cancer/ Pancreatic Cancer/ Bladder Cancer/ Colorectal Cancer	Oricol Test	All	NA	2021-12
18.	NCT01038492	p16 Methylation for Smoking Cessation	Tobacco Use Disorder/ Smoking Cessation/ Lung Cancer	p16 Methylation and Lung Cancer Education	All	NA	2009-04
19.	NCT00978250	A Multi-Histology Phase II Study of 5-Fluoro-2'-Deoxycytidine With Tetrahydrouridine (FdCyd + THU)	Head and Neck Neoplasms/ Lung Neoplasms/ Urinary Bladder Neoplasms/ Breast Neoplasms	5-Fluoro-2'-Deoxycytidine (FdCyd) + Tetrahydrouridine (THU)	All	II	2009-08
20.	NCT02489903	RRx-001 in Lung Cancer, Ovarian Cancer and Neuroendocrine Tumors Prior to Re-administration of Platinum Based Doublet Regimens (QUADRUPLE THREAT)	Small Cell Carcinoma/ Non-Small-Cell Lung/ Neuroendocrine Tumors/ Ovarian Epithelial Cancer	RRx-001, Cisplatin, Etoposide, Carboplatin, Irinotecan, Vinorelbine, Doxil, Gemcitabine, Taxane, Paclitaxel, Nab-Paclitaxel, Pemetrexed	All	II	2015-06

more research is required to identify robust and reliable epigenetic markers that can accurately predict immunotherapy responses, especially in patients with a “cold” immunophenotype.^{15,24} Comprehensive genome-wide approaches and large-scale validation studies are essential for uncovering novel biomarkers. Second, the development of epigenetic-targeted therapies holds great potential for enhancing treatment responses. Targeting specific epigenetic alterations, such as DNAm patterns, may help modulate the tumor microenvironment and sensitize tumors to immunotherapy.⁹⁰ However, the development of effective and safe epigenetic therapies remains a complex challenge. Additionally, incorporating cell-

free DNAm analysis into non-invasive diagnostic methods, such as liquid biopsies, is a promising avenue.⁹¹ This approach can improve the accuracy of early cancer detection and monitoring of treatment responses, ultimately advancing personalized medicine.

However, challenges such as the standardization of analytical methods, addressing sample heterogeneity, and refining the clinical utility of epigenetic biomarkers must be overcome. Furthermore, understanding the intricate interplay between immune-related adverse events and DNAm patterns in lung cancer cells is crucial for improving patient care and mitigating the side effects associated with immunotherapy.⁹² In conclusion, although epigenetic biomarkers and epigenetic-targeted therapies offer significant potential for improving lung cancer treatment, continued research, collaboration, and innovation are essential to fully realize their clinical impact and enhance patient outcomes.

Conclusion

Among the diverse biomarkers explored for predicting immunotherapy responses in lung cancer, DNAm has emerged as one of the most promising and clinically relevant indicators. Its stability, early involvement in tumorigenesis, and strong correlation with immune activation make it uniquely suited for diagnostic and predictive applications. Aberrant methylation patterns influence key processes, including antigen presentation, interferon signaling, immune evasion, and tumor phenotypes. These changes not only shape intrinsic tumor biology but also modulate sensitivity to immune checkpoint inhibitors. Importantly, DNAm can be reliably detected through minimally invasive methods such as blood-based liquid biopsies, offering real-time monitoring of disease progression and treatment response. Although established biomarkers, such as PD-L1, TMB, and MSI provide valuable but incomplete insights, integrating DNAm signatures with these existing markers creates a more comprehensive and precise framework for patient selection. As multi-omics approaches continue to evolve, methylation-based biomarkers are expected to play a central role in advancing personalized immunotherapy. Continued validation in large patient cohorts and standardization of methylation-based assays are essential steps toward incorporating these powerful epigenetic markers into routine clinical practice, ultimately improving the outcomes of patients with lung cancer.

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