Immunotherapy in Non–Small Cell Lung Cancer: Mechanisms, Biomarkers, Clinical Outcomes, and Future Directions

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Abstract

Background: Immunotherapy has transformed the therapeutic landscape of non–small cell lung cancer (NSCLC), redefining standards of care across metastatic, locally advanced, and early-stage disease. Historically associated with poor survival outcomes, advanced NSCLC management was dominated by platinum-based chemotherapy with limited durability of response. The introduction of immune checkpoint inhibitors targeting programmed death-1 (PD-1) and programmed death-ligand 1 (PD-L1) has resulted in significant improvements in overall survival, progression-free survival, and long-term disease control in selected patient populations. These therapies restore antitumor immune responses by reversing tumor-induced T-cell exhaustion, thereby shifting treatment paradigms from cytotoxic eradication to immune modulation. Pivotal clinical trials established PD-1/PD-L1 inhibitors as first-line therapy in PD-L1–high metastatic NSCLC and as backbone components of chemo-immunotherapy combinations regardless of PD-L1 status. Furthermore, consolidation immunotherapy following definitive chemoradiotherapy has significantly improved survival in unresectable stage III disease. More recently, neoadjuvant and adjuvant immunotherapy strategies have demonstrated promising results in resectable NSCLC, expanding the role of immune-based therapies into earlier disease stages. Despite these advances, therapeutic benefit remains heterogeneous. Predictive biomarkers such as PD-L1 expression, tumor mutational burden, and genomic co-alterations only partially explain response variability. Mechanisms of primary and acquired resistance, immune-related toxicities, and optimal treatment sequencing remain active areas of investigation. Moreover, special populations—including patients with oncogene-driven tumors or brain metastases—pose unique challenges in immunotherapy integration. This review comprehensively examines the biological rationale for immunotherapy in NSCLC, summarizes key clinical trial evidence across disease stages, evaluates current and emerging predictive biomarkers, explores resistance mechanisms, and discusses future therapeutic directions. A multidimensional understanding integrating tumor biology, immune contexture, and clinical evidence is essential to optimize precision immuno-oncology strategies in NSCLC.

Keywords: *Immunotherapy, Non–Small Cell Lung Cancer, Future Directions*

Introduction

Lung cancer remains the leading cause of cancer-related mortality worldwide, with non–small cell lung cancer (NSCLC) accounting for approximately 85% of all cases [1]. For decades, treatment of advanced NSCLC relied primarily on platinum-based chemotherapy, yielding modest response rates and limited survival improvement. The recognition that lung tumors actively suppress host immune surveillance mechanisms provided the conceptual foundation for immunotherapeutic intervention, marking a paradigm shift in thoracic oncology [2].

Tumor cells evade immune destruction through multiple mechanisms, including upregulation of immune checkpoint pathways such as PD-1 and its ligand PD-L1. Binding of PD-L1 expressed on tumor cells to PD-1 receptors on T lymphocytes induces T-cell exhaustion and functional anergy, impairing cytotoxic antitumor responses. Immune checkpoint inhibitors disrupt this inhibitory signaling axis, restoring immune-mediated tumor recognition and elimination [3].

The clinical relevance of checkpoint blockade in NSCLC was first demonstrated in large randomized trials comparing PD-1 inhibitors to docetaxel in previously treated metastatic disease. These studies revealed significant improvements in overall survival, with a subset of patients achieving durable responses exceeding several years—an outcome rarely observed with chemotherapy alone [4,5]. Subsequent trials established pembrolizumab monotherapy as first-line treatment for patients with high PD-L1 expression, demonstrating superior survival compared with platinum-based chemotherapy [6].

Chemo-immunotherapy combinations further broadened therapeutic applicability by improving outcomes regardless of PD-L1 expression status. Trials evaluating pembrolizumab combined with platinum doublet chemotherapy showed significant survival benefit in both squamous and nonsquamous histologies, reinforcing immunotherapy as a foundational component of metastatic NSCLC management [7,8]. These results signaled the transition of immunotherapy from second-line salvage therapy to first-line standard of care.

The role of immunotherapy expanded beyond metastatic disease with the PACIFIC trial, which demonstrated improved progression-free and overall survival with durvalumab consolidation following concurrent chemoradiotherapy in unresectable stage III NSCLC [9]. More recently, neoadjuvant and adjuvant immunotherapy strategies have shown promising event-free survival benefits in resectable disease, suggesting immune modulation may enhance curative-intent treatment outcomes [10].

Despite these transformative advances, substantial challenges remain. A significant proportion of patients do not respond to immunotherapy, and predictive biomarkers remain imperfect. PD-L1 expression, while clinically useful, is limited by assay variability and tumor heterogeneity. Tumor mutational burden, immune gene signatures, and co-mutation patterns provide additional insights but lack universal standardization [11].

Furthermore, oncogene-driven NSCLC—particularly EGFR- or ALK-altered tumors—exhibits limited responsiveness to checkpoint inhibition, underscoring the complexity of tumor–immune interactions [12]. Immune-related adverse events, including pneumonitis, colitis, endocrinopathies, and myocarditis, also require careful management, especially in patients receiving multimodality therapy [13].

Therefore, the aim of this review is to critically evaluate the mechanistic basis, clinical trial evidence, predictive biomarkers, resistance mechanisms, and future therapeutic strategies of immunotherapy in NSCLC. By integrating biological insights with clinical data, we seek to identify current research gaps and propose directions for optimizing precision immuno-oncology in this heterogeneous disease.

Biological Mechanisms of Immune Checkpoint Inhibition in NSCLC

The immune system continuously surveys tissues for malignant transformation through a process known as cancer immunosurveillance. In NSCLC, chronic tobacco exposure and environmental carcinogens generate high mutational burdens, leading to the formation of neoantigens capable of eliciting T-cell–mediated immune responses. However, tumor cells evolve adaptive mechanisms to evade immune destruction, establishing a dynamic equilibrium described as cancer immunoediting, encompassing

elimination, equilibrium, and escape phases [14].

One of the principal immune escape mechanisms in NSCLC involves the PD-1/PD-L1 axis. Programmed death-1 (PD-1) is an inhibitory receptor expressed on activated T cells, while PD-L1 is frequently upregulated on tumor cells and tumor-associated immune cells. Engagement of PD-1 by PD-L1 leads to suppression of T-cell receptor signaling, decreased cytokine production, and impaired cytotoxic function. This interaction induces T-cell exhaustion, characterized by diminished proliferative capacity and effector activity, thereby allowing tumor persistence despite antigen recognition [15].

PD-L1 expression in NSCLC may arise through two distinct mechanisms: constitutive oncogenic signaling and adaptive immune resistance. Oncogenic pathways such as EGFR activation, ALK rearrangements, and PI3K/AKT signaling can intrinsically upregulate PD-L1 expression. Conversely, inflammatory cytokines—particularly interferon-γ released by activated T cells—can induce PD-L1 expression as part of an adaptive feedback mechanism. This dual regulatory pathway underscores the complexity of interpreting PD-L1 as a biomarker and highlights the interplay between tumor genetics and immune microenvironment [16].

Cytotoxic T-lymphocyte–associated protein 4 (CTLA-4) represents another immune checkpoint that regulates early T-cell activation within lymphoid tissues. CTLA-4 competes with the co-stimulatory receptor CD28 for binding to B7 ligands on antigen-presenting cells, thereby attenuating T-cell priming. Although CTLA-4 inhibition has demonstrated greater impact in melanoma, combination strategies targeting both PD-1 and CTLA-4 have shown activity in NSCLC, particularly in patients with high tumor mutational burden [17].

Tumor mutational burden (TMB) contributes mechanistically to immunotherapy responsiveness by increasing the repertoire of neoantigens presented on major histocompatibility complex (MHC) molecules. High TMB enhances tumor immunogenicity, facilitating T-cell recognition and immune activation. Smoking-associated NSCLC often exhibits elevated TMB, partially explaining improved responses to immune checkpoint inhibitors compared with oncogene-driven tumors, which generally harbor lower mutational burdens [18].

Effective antitumor immunity requires intact antigen presentation machinery. Tumor cells process intracellular proteins into peptide fragments that are presented on MHC class I molecules for recognition by CD8+ T cells. Disruptions in antigen presentation—such as loss of β2-microglobulin or mutations affecting MHC expression—can impair immune recognition and contribute to primary or acquired resistance to checkpoint blockade [19].

The tumor microenvironment (TME) further shapes immunotherapy outcomes through complex cellular interactions. Tumor-associated macrophages, regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), and cancer-associated fibroblasts may establish an immunosuppressive milieu that inhibits cytotoxic T-cell infiltration and function. The balance between effector immune cells and suppressive populations determines whether the TME is inflamed (“hot”) or non-inflamed (“cold”), influencing responsiveness to immune checkpoint inhibition [20].

Interferon signaling plays a pivotal role in mediating immunotherapy efficacy. Activation of interferon-γ pathways enhances antigen presentation, promotes chemokine production, and facilitates T-cell recruitment. However, chronic interferon signaling may also induce adaptive resistance mechanisms, including upregulation of alternative immune checkpoints and immunosuppressive cytokines. Thus, interferon signaling exerts context-dependent effects within the tumor-immune interface [21].

Metabolic competition within the TME represents an additional regulatory mechanism. Rapidly proliferating tumor cells consume glucose and oxygen, creating hypoxic and nutrient-deprived conditions that impair T-cell function. Accumulation of lactate and adenosine within the TME further suppresses immune activity. These metabolic constraints highlight potential therapeutic opportunities combining checkpoint inhibition with metabolic modulators [22].

Radiotherapy and chemotherapy may enhance immunotherapy responses through immunogenic cell death. Cytotoxic therapies can increase neoantigen release, promote dendritic cell activation, and upregulate PD-L1 expression, thereby providing a mechanistic rationale for chemo-immunotherapy and

radio-immunotherapy combinations. This synergy underpins the success of combination regimens in both metastatic and locally advanced NSCLC [23].

In oncogene-driven NSCLC, such as EGFR-mutant or ALK-rearranged tumors, the immune microenvironment often demonstrates reduced T-cell infiltration and lower TMB, contributing to diminished responsiveness to checkpoint blockade. Additionally, specific oncogenic pathways may directly modulate immune signaling networks. Understanding these mechanistic distinctions is critical when integrating immunotherapy with targeted therapies [24].

Collectively, the biological mechanisms underlying immune checkpoint inhibition in NSCLC reflect a complex interplay between tumor genomics, antigenicity, immune regulation, and microenvironmental context. Effective immunotherapy depends not only on blocking inhibitory pathways but also on maintaining adequate antigen presentation, T-cell priming, and immune infiltration. A mechanistic understanding of these processes provides the foundation for rational biomarker development and novel combinatorial strategies aimed at overcoming resistance and enhancing durable clinical benefit [25].

Immune Checkpoint Inhibitors in Metastatic NSCLC: First-Line Monotherapy and Chemo-Immunotherapy Paradigms

The introduction of immune checkpoint inhibitors (ICIs) in metastatic NSCLC represents one of the most transformative milestones in thoracic oncology. Early phase III trials demonstrated that PD-1 blockade improved overall survival compared with docetaxel in previously treated patients, establishing immunotherapy as standard second-line therapy. These findings were remarkable not only for survival benefit but also for the durability of responses observed in a subset of patients, suggesting the possibility of long-term disease control [26].

The transition of immunotherapy into the first-line setting was driven by the KEYNOTE-024 trial, which evaluated pembrolizumab monotherapy versus platinum-based chemotherapy in patients with PD-L1 tumor proportion score (TPS) ≥50%. Pembrolizumab significantly improved progression-free survival and overall survival, with a more favorable toxicity profile. This trial established PD-L1 expression as a clinically actionable biomarker guiding frontline monotherapy selection [27].

Subsequent trials expanded the PD-L1 threshold. KEYNOTE-042 included patients with PD-L1 TPS ≥1% and demonstrated overall survival benefit with pembrolizumab monotherapy compared with chemotherapy, although the magnitude of benefit was greatest in patients with PD-L1 ≥50%. These findings underscored the graded predictive value of PD-L1 expression and highlighted the need to interpret intermediate expression levels cautiously [28].

Despite the success of monotherapy in PD-L1–high tumors, many patients present with low or negative PD-L1 expression. Combination chemo-immunotherapy strategies were developed to broaden therapeutic benefit. The KEYNOTE-189 trial demonstrated that pembrolizumab combined with platinum–pemetrexed chemotherapy significantly improved overall survival in metastatic nonsquamous NSCLC regardless of PD-L1 expression status. Importantly, survival benefit was observed even in PD-L1–negative tumors, suggesting chemotherapy may enhance immunogenicity and synergize with checkpoint inhibition [29].

Similarly, KEYNOTE-407 evaluated pembrolizumab in combination with carboplatin and either paclitaxel or nab-paclitaxel in squamous NSCLC. The addition of pembrolizumab significantly improved overall survival and progression-free survival compared with chemotherapy alone. This trial extended chemo-immunotherapy benefit to squamous histology, confirming the broad applicability of combination strategies across NSCLC subtypes [30].

Atezolizumab-based regimens further diversified treatment options. The IMpower150 trial evaluated atezolizumab in combination with bevacizumab and chemotherapy in nonsquamous NSCLC and demonstrated improved survival compared with bevacizumab plus chemotherapy. Notably, this regimen showed activity even in patients with EGFR mutations after targeted therapy failure, suggesting a potential role for anti-angiogenic modulation in enhancing immunotherapy efficacy [31].

Dual immune checkpoint blockade has also been explored in metastatic NSCLC. The CheckMate 227 trial evaluated nivolumab plus ipilimumab and demonstrated improved overall survival compared with

chemotherapy, particularly in patients with high tumor mutational burden. Although TMB did not ultimately become a routine biomarker in this context, dual immunotherapy offered a chemotherapy-free option for selected patients [32].

The CheckMate 9LA regimen combined nivolumab and ipilimumab with limited cycles of chemotherapy, aiming to provide rapid disease control while establishing durable immune response. This approach demonstrated improved overall survival compared with chemotherapy alone, further supporting flexible integration of chemotherapy and dual immunotherapy in frontline treatment [33].

Long-term follow-up of immunotherapy trials has revealed a characteristic survival plateau in a subset of patients, suggesting durable immune-mediated tumor control. Five-year survival rates in PD-L1–high populations treated with pembrolizumab monotherapy substantially exceed historical chemotherapy outcomes. These durable responses represent a defining feature distinguishing immunotherapy from cytotoxic regimens [34].

However, therapeutic selection must account for molecular subtypes. Patients harboring EGFR mutations or ALK rearrangements derive limited benefit from single-agent PD-1/PD-L1 inhibitors. In these populations, targeted therapy remains the preferred initial approach, with immunotherapy considered cautiously after exhaustion of targeted options [35].

Brain metastases, present in a significant proportion of metastatic NSCLC patients, represent another critical clinical consideration. Immune checkpoint inhibitors have demonstrated intracranial activity, particularly in PD-L1–positive tumors, although outcomes are influenced by symptom burden and steroid use. Integration with radiotherapy may further enhance intracranial control through potential synergistic immune effects [36].

Safety profiles of ICIs differ substantially from chemotherapy. Immune-related adverse events (irAEs) such as pneumonitis, thyroid dysfunction, colitis, hepatitis, and dermatologic reactions arise from immune activation rather than cytotoxic injury. Although generally manageable with corticosteroids and immunosuppressive therapy, severe irAEs may require permanent discontinuation. Early recognition and multidisciplinary management are critical to maintaining therapeutic benefit [37].

Collectively, first-line immunotherapy strategies in metastatic NSCLC now include monotherapy for PD-L1–high tumors, chemo-immunotherapy combinations for broader populations, and dual immunotherapy regimens for selected patients. Treatment selection depends on PD-L1 expression, tumor burden, symptomatology, molecular alterations, and patient comorbidities. The evolution of these paradigms reflects a shift toward personalized immune-based therapy grounded in biological and clinical stratification [38].

Immunotherapy in Locally Advanced (Stage III) NSCLC: The PACIFIC Paradigm and Beyond

Unresectable stage III NSCLC represents a biologically heterogeneous group of tumors characterized by locoregional advancement without distant metastasis. Historically, concurrent chemoradiotherapy (CRT) was the standard of care, achieving median overall survival of approximately 28–30 months, with high rates of locoregional and distant relapse. Despite optimal CRT, durable long-term survival remained limited, underscoring the need for consolidation strategies capable of enhancing systemic immune surveillance and preventing metastatic dissemination [39].

The PACIFIC trial marked a paradigm shift in the management of unresectable stage III NSCLC. This randomized phase III study evaluated durvalumab, an anti–PD-L1 antibody, as consolidation therapy following definitive concurrent CRT in patients without disease progression. Durvalumab significantly improved progression-free survival compared with placebo, with a median PFS of 16.8 months versus 5.6 months. This trial provided the first evidence that immunotherapy could meaningfully improve outcomes in locally advanced NSCLC following CRT [40].

Updated overall survival data from PACIFIC confirmed sustained benefit, with a significant improvement in median overall survival and a notable increase in 5-year survival rates. Importantly, the survival advantage was observed across most predefined subgroups, solidifying consolidation durvalumab as the new standard of care after concurrent CRT. The trial also demonstrated durable disease control, reflecting the capacity of immunotherapy to establish long-term immune-mediated

tumor suppression [41].

The biological rationale for post-CRT immunotherapy is grounded in radiation-induced immunogenic modulation. Radiotherapy can enhance antigen presentation, increase PD-L1 expression, promote dendritic cell activation, and induce immunogenic cell death. This process may convert tumors into a more inflamed phenotype, thereby enhancing susceptibility to PD-L1 blockade. The synergy between radiotherapy and immunotherapy supports the concept of CRT as an immune-priming platform [42].

Subgroup analyses of the PACIFIC trial suggested that PD-L1 expression may influence magnitude of benefit, although durvalumab demonstrated activity irrespective of PD-L1 status. Regulatory differences emerged internationally, with some regions restricting use based on PD-L1 expression thresholds. However, real-world data have generally supported broad applicability in patients without progression after CRT, emphasizing clinical benefit beyond strict biomarker stratification [43].

Timing of immunotherapy initiation following CRT appears clinically relevant. In PACIFIC, durvalumab was initiated within 42 days after completion of CRT. Subsequent analyses suggested that earlier initiation may correlate with improved outcomes, potentially reflecting heightened immune activation during the post-radiation window. Ongoing studies continue to explore optimal sequencing and timing strategies [44].

Safety considerations are particularly important in the stage III setting. Pneumonitis represents a significant adverse event risk due to overlapping toxicities from thoracic radiotherapy and immune checkpoint inhibition. In PACIFIC, rates of grade 3 or 4 pneumonitis were relatively low, and treatment discontinuation due to pneumonitis was manageable. Careful patient selection and vigilant monitoring are essential to balance therapeutic benefit against pulmonary toxicity [45].

Beyond PACIFIC, investigations are exploring concurrent rather than sequential immunotherapy with CRT. Early-phase trials evaluating PD-1/PD-L1 inhibitors administered during chemoradiation suggest feasibility, though definitive survival data are pending. The theoretical advantage lies in simultaneous immune activation and antigen release; however, concerns regarding additive toxicity remain under evaluation [46].

Neoadjuvant immunotherapy in potentially resectable stage III disease is another rapidly evolving domain. Trials assessing immune checkpoint inhibitors administered prior to surgery have demonstrated promising rates of major pathologic response, suggesting that early immune activation may eradicate micrometastatic disease and enhance long-term survival. These findings blur the traditional boundary between locally advanced and operable disease paradigms [47].

The integration of consolidation immunotherapy has redefined survival expectations in unresectable stage III NSCLC. Five-year survival rates now approach those historically observed only in earlier-stage disease, underscoring the transformative impact of immune-based strategies. Nonetheless, relapse still occurs in a substantial proportion of patients, indicating persistent challenges related to immune escape and resistance mechanisms [48].

Future directions in stage III disease include biomarker refinement, incorporation of dual immune checkpoint blockade, and integration of novel immunomodulatory agents. Circulating tumor DNA (ctDNA) assessment following CRT may identify minimal residual disease and refine patient selection for intensified immunotherapy approaches. Such strategies aim to personalize treatment intensity while minimizing unnecessary toxicity [49].

In summary, the PACIFIC paradigm established consolidation PD-L1 blockade as the standard of care following definitive concurrent chemoradiotherapy in unresectable stage III NSCLC. The mechanistic synergy between radiation-induced immunogenicity and immune checkpoint inhibition provides a compelling biological framework. Continued research will determine whether earlier integration, combination strategies, or biomarker-guided approaches can further improve long-term survival in this complex disease stage [50].

Immunotherapy in Early-Stage NSCLC: Neoadjuvant and Adjuvant Strategies

Surgical resection remains the cornerstone of treatment for early-stage NSCLC; however, recurrence rates remain substantial, particularly in stage II–IIIA disease. Historically, platinum-based adjuvant

chemotherapy provided modest absolute survival benefit of approximately 5% at five years, underscoring the need for more effective perioperative strategies. The recognition that micrometastatic disease often persists after surgery has driven interest in immune-based approaches aimed at enhancing systemic tumor control in the curative-intent setting [51].

Neoadjuvant immunotherapy offers several theoretical advantages. Administering immune checkpoint inhibitors prior to tumor resection allows exposure of the immune system to the intact tumor and its full neoantigen repertoire, potentially enhancing T-cell priming and clonal expansion. Additionally, neoadjuvant therapy provides an opportunity to evaluate pathological response as an early surrogate endpoint of efficacy. These biological considerations have fueled rapid clinical investigation of preoperative immunotherapy regimens [52].

Early phase studies evaluating neoadjuvant nivolumab monotherapy demonstrated encouraging rates of major pathologic response (MPR), defined as ≤10% viable tumor cells in the resected specimen. Importantly, radiographic responses did not always correlate with pathological responses, highlighting the unique response patterns associated with immunotherapy. These initial findings established proof of concept that immune checkpoint blockade could induce meaningful tumor regression prior to surgery [53].

The phase III CheckMate 816 trial further advanced this strategy by evaluating nivolumab in combination with platinum-based chemotherapy in resectable stage IB–IIIA NSCLC. The addition of nivolumab significantly improved event-free survival and increased the rate of pathological complete response (pCR) compared with chemotherapy alone. These results established neoadjuvant chemo-immunotherapy as a new standard option for selected patients with resectable disease [47].

Importantly, the benefit observed in CheckMate 816 was seen across PD-L1 subgroups, though greater magnitude of response was noted in patients with higher PD-L1 expression. The trial also demonstrated that neoadjuvant immunotherapy did not compromise surgical feasibility or increase perioperative morbidity. These findings alleviated initial concerns regarding immune-related inflammation complicating surgical procedures [47].

Adjuvant immunotherapy has also reshaped postoperative management. The IMpower010 trial evaluated atezolizumab following adjuvant chemotherapy in resected stage II–IIIA NSCLC. Atezolizumab significantly improved disease-free survival in patients with PD-L1–positive tumors, particularly those with PD-L1 expression ≥1%. These results supported regulatory approval of adjuvant PD-L1 blockade in selected populations [67].

Similarly, the KEYNOTE-091 (PEARLS) trial assessed adjuvant pembrolizumab in resected stage IB–IIIA NSCLC, demonstrating improvement in disease-free survival irrespective of PD-L1 expression, though magnitude varied by subgroup. These findings suggest that immune checkpoint inhibition may reduce recurrence risk even in patients with lower PD-L1 expression, although biomarker-driven refinement remains an area of active investigation [54].

The integration of targeted therapy into the perioperative setting adds complexity to treatment selection. The ADAURA trial demonstrated substantial disease-free survival benefit with adjuvant osimertinib in resected EGFR-mutant NSCLC. Consequently, in patients with actionable driver mutations, targeted therapy often supersedes immunotherapy in the adjuvant setting. Molecular testing is therefore essential in determining optimal postoperative strategy [10].

Circulating tumor DNA (ctDNA) analysis has emerged as a promising biomarker for minimal residual disease (MRD) detection after surgery. Detection of ctDNA following resection correlates strongly with recurrence risk and may identify patients who derive the greatest benefit from adjuvant immunotherapy. Incorporation of MRD assessment into clinical trials may enable more personalized perioperative treatment intensification [49].

Neoadjuvant and adjuvant immunotherapy raise important questions regarding optimal sequencing and duration. Ongoing trials are evaluating perioperative regimens combining both pre- and postoperative immunotherapy to maximize systemic immune activation. The balance between efficacy, toxicity, and cost-effectiveness remains under evaluation as longer-term survival data mature [55].

Immune-related adverse events in the perioperative setting warrant careful monitoring. While most events are manageable, endocrine toxicities such as thyroid dysfunction may be permanent. The risk–benefit ratio must be carefully weighed in patients with potentially curable disease, emphasizing the importance of multidisciplinary coordination between oncologists, thoracic surgeons, and pulmonologists [37].

Collectively, immunotherapy has expanded into early-stage NSCLC, fundamentally altering the perioperative treatment paradigm. Neoadjuvant chemo-immunotherapy and adjuvant PD-1/PD-L1 blockade have demonstrated meaningful reductions in recurrence risk and improved event-free survival. Future research will clarify long-term overall survival impact, refine biomarker-driven patient selection, and determine whether integrated perioperative strategies can further enhance cure rates in resectable NSCLC [56].

Predictive Biomarkers in Immunotherapy for NSCLC: PD-L1, TMB, Co-Mutations, and Emerging Molecular Signatures

PD-L1 expression by immunohistochemistry (IHC) remains the most widely used predictive biomarker for selecting immune checkpoint inhibitors in NSCLC, particularly for first-line pembrolizumab monotherapy. Its clinical utility is strongest at higher thresholds (eg, TPS ≥50%), yet PD-L1 is an imperfect surrogate for antitumor immunity because it reflects only one adaptive immune-resistance mechanism and can vary across tumor regions and over time. Consequently, PD-L1 is best interpreted as a probability marker rather than a definitive classifier of response versus resistance, especially when treatment choices include chemo-immunotherapy regardless of PD-L1 status [68].

A major practical limitation of PD-L1 as a biomarker is assay and platform variability. Different antibody clones and companion diagnostic assays (eg, 22C3, 28-8, SP263, SP142) have differing analytical performance, staining characteristics, and scoring conventions across tumor and immune cells. Comparative harmonization studies (eg, Blueprint) demonstrated that several assays show broadly comparable tumor-cell staining, whereas others may stain fewer tumor cells, with direct implications for treatment eligibility when rigid cutoffs are applied. This variability underlines the importance of laboratory validation, pathologist training, and standardized pre-analytic handling to reduce misclassification risk [69].

Spatial and temporal heterogeneity further complicate PD-L1 interpretation. PD-L1 expression may differ between primary tumors and metastatic deposits, and small biopsies may not represent the full immune landscape. This is clinically relevant because treatment decisions are often based on limited tissue obtained at diagnosis, while the immune microenvironment may evolve under therapy or during progression. Hence, discordance between sampled tissue and the “true” global tumor PD-L1 state contributes to both false-negative and false-positive clinical predictions [70].

Tumor mutational burden (TMB) has been investigated as a proxy for neoantigen load and immunogenicity, based on the concept that higher numbers of nonsynonymous mutations generate more recognizable neoepitopes. In NSCLC, smoking-associated tumors tend to have higher TMB, aligning with higher response rates observed in some immunotherapy cohorts. However, despite biological plausibility and supportive trial-level associations, the clinical implementation of TMB has been constrained by platform variability, differences in bioinformatic pipelines, and inconsistent thresholds across assays and studies [71].

Gene-expression signatures representing an “inflamed” tumor microenvironment provide a complementary approach that captures immune activation rather than tumor genomics alone. Interferon-γ–related expression profiles and T-cell–inflamed gene signatures have been associated with response to PD-1 blockade across multiple tumor types, reflecting pre-existing immune engagement and antigen presentation. In NSCLC, these transcriptomic markers may help discriminate PD-L1–positive but immunologically inactive tumors from those with coordinated immune activation, supporting the rationale for composite biomarker models [72].

Tumor-infiltrating lymphocytes (TILs), particularly CD8+ cytotoxic T cells and their spatial localization (intratumoral vs stromal), are strongly linked to immunotherapy responsiveness because checkpoint

blockade depends on reinvigorating antigen-experienced T cells. High CD8 infiltration and an immune-inflamed phenotype generally correlate with improved response and survival, while immune-excluded tumors demonstrate peripheral immune presence without effective tumor penetration. Standardized quantitative pathology and emerging multiplex immunofluorescence approaches are increasingly used to refine immune-context assessment beyond PD-L1 alone [73].

Co-occurring genomic alterations can markedly modulate immunotherapy benefit, especially within KRAS-mutant lung adenocarcinoma. STK11 (LKB1) alterations have been repeatedly associated with an immune-cold microenvironment, reduced T-cell infiltration, and inferior outcomes to PD-1/PD-L1 blockade, even when PD-L1 is expressed. These observations are clinically important because they explain resistance in subsets that might otherwise appear eligible by PD-L1 criteria and support integrating co-mutation context into predictive frameworks [74].

Similarly, KEAP1/NFE2L2 pathway alterations have been linked to aggressive biology and reduced benefit from immunotherapy in several real-world and trial-adjacent analyses. Mechanistically, altered oxidative stress responses and metabolic reprogramming may contribute to immune evasion and therapy resistance. While KEAP1 is not yet a routine exclusion biomarker, its consistent association with poorer outcomes suggests it should be considered when interpreting expected benefit, particularly in KRAS-driven disease treated with checkpoint inhibitors [75].

Oncogene-driven NSCLC (notably EGFR-mutant and ALK-rearranged tumors) generally exhibits lower TMB, less inflamed microenvironments, and limited responsiveness to checkpoint inhibitors compared with smoking-associated NSCLC. Clinically, these tumors have demonstrated lower response rates and shorter benefit duration with PD-1/PD-L1 inhibitors, shaping guideline-based sequencing in which targeted therapy remains the preferred initial approach. This context reinforces the principle that predictive biomarkers must be integrated with oncogenic subtype rather than applied uniformly across NSCLC [76].

Circulating tumor DNA (ctDNA) is emerging as a dynamic biomarker that can complement tissue-based predictors. In advanced disease, early on-treatment ctDNA decline has been associated with response and improved outcomes in immunotherapy-treated patients, reflecting real-time tumor burden changes. In curative-intent settings, postoperative ctDNA positivity (molecular residual disease) identifies high recurrence risk and may help select patients most likely to benefit from adjuvant immunotherapy intensification, supporting an individualized escalation/de-escalation strategy [77].

The gut microbiome has also been implicated as a host-level predictor of immunotherapy response, potentially through modulation of systemic immunity and antigen cross-reactivity. Antibiotic exposure around immunotherapy initiation has been associated with reduced efficacy in several analyses, and specific microbial signatures have correlated with improved outcomes in checkpoint blockade cohorts. While not ready for routine clinical deployment in NSCLC, this domain highlights the broader concept that predictive modeling may need to incorporate host factors alongside tumor biomarkers [78].

Taken together, predictive biomarker development in NSCLC immunotherapy is shifting from single-marker selection (PD-L1 alone) toward multidimensional models integrating tumor-cell features (PD-L1, antigen presentation), tumor genomics (TMB, co-mutations), immune context (TILs, gene signatures), dynamic blood-based measures (ctDNA), and host modifiers (microbiome, inflammation). The central research gap remains biomarker standardization and prospective validation of composite algorithms that are practical, reproducible, and treatment-actionable across global clinical settings [79].

Conclusion

Immunotherapy has redefined the treatment paradigm of non–small cell lung cancer (NSCLC) across all disease stages. Immune checkpoint inhibitors targeting the PD-1/PD-L1 axis have significantly improved survival in metastatic disease, established consolidation therapy as standard after chemoradiotherapy in stage III disease, and expanded into neoadjuvant and adjuvant settings in resectable tumors. The durability of responses and long-term survival plateaus observed in a subset of patients distinguish immunotherapy from traditional cytotoxic approaches.

Despite these advances, therapeutic benefit remains heterogeneous. PD-L1 expression, tumor mutational

burden, immune gene signatures, and co-mutation profiles provide predictive insight but lack sufficient precision when used independently. Tumor heterogeneity, dynamic immune interactions, and oncogenic context continue to limit universal responsiveness, particularly in driver-mutated tumors.

Future progress depends on integrating multidimensional biomarkers, refining patient selection, and developing rational combination strategies to overcome primary and acquired resistance. As immunotherapy continues to move earlier in the disease course, balancing efficacy with toxicity becomes increasingly important. Ultimately, precision immuno-oncology aims not only to extend survival but to achieve durable disease control—and potentially cure—in a broader population of patients with NSCLC.

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