Predictive Value and Limitations of Biomarkers for PD-1/PD-L1 Checkpoint Inhibitor Therapy in Non–Small Cell Lung Cancer

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Contents

1 Abstract

Background

Immune checkpoint inhibitor therapy targeting the PD-1/PD-L1 pathway have significantly improved outcomes for a subset of patients with advanced non-small cell lung cancer (NSCLC). However, only a minority of patients derive durable benefit, while treatment is associated with substantial toxicity and cost. Reliable predictive biomarkers are therefore essential to optimize patient selection.

Objective

This literature review evaluates the predictive value of established and emerging biomarkers for response to PD-1/PD-L1 checkpoint inhibitor therapy in NSCLC.

Methods

A structured review of the literature was performed focusing on tissue-based biomarkers, including PD-L1 expression, Tumor Mutational Burden (TMB), and Microsatellite Instability (MSI), as well as circulating biomarkers such as circulating tumor DNA (ctDNA) and cytokines.

Results

PD-L1 expression is the most widely used biomarker and enriches for responders, particularly at high expression levels, but demonstrates limited sensitivity and specificity due to biological heterogeneity and assay variability. TMB reflects tumor mutational load and potential neoantigen generation but shows inconsistent predictive performance and is confounded by factors such as smoking status and immune activation. MSI is a strong predictor of response to PD-1 blockade but occurs very rarely in NSCLC, limiting its clinical utility. Circulating biomarkers, particularly dynamic changes in ctDNA during treatment, are strongly associated with clinical outcomes but primarily reflect treatment response rather than baseline sensitivity.

Conclusion

No single biomarker reliably predicts response to PD-1/PD-L1 therapy in NSCLC. Current evidence supports a shift toward integrated biomarker strategies that combine tumor immune contexture, antigen presentation capacity, and dynamic response monitoring. Prospective validation of such multidimensional approaches is required to improve patient selection and clinical outcomes.

2 Introduction

Lung cancer is the leading cause of cancer-related mortality, with a 5-year relative survival rate of 25% [1]. Non-small cell lung cancer (NSCLC) is the most common type of lung cancer. Each year in the Netherlands there are approximately 14,500 lung cancer patients. 70% are diagnosed with NSCLC [2, 3]. Despite advances in surgery, chemotherapy, and targeted therapy, the prognosis for patients with advanced non-small cell lung cancer remains poor, with a median overall survival of approximately 8-12 months in the pre-immunotherapy era and only modest improvements with conventional systemic therapies [4]. Over the past decade there has been substantial progress in improving survival through the use of ICI therapy, particularly antibody-based therapies targeting the programmed cell death 1 (PD-1) receptor and its ligand PD-L1. These antibodies can induce a durable response by restoring antitumor immune activity [5, 6, 7]. Currently, most patients with advanced NSCLC receive PD-1 or PD-L1 checkpoint inhibitors in combination with chemotherapy in the first-line treatment setting [4].

The PD-1/PD-L1 pathway plays a crucial role in regulating the immune response. Normally, the interaction between the PD-1 receptor on activated T-cells and its ligand PD-L1, which is expressed on tumor cells and other immune cells, helps maintain immune tolerance and prevents excessive immune activation. However, many tumor cells exploit this mechanism by overexpressing PD-L1, which suppresses T-cell activity and allows the tumor to evade immune destruction. PD-1/PD-L1 checkpoint inhibitors, such as nivolumab and pembrolizumab (anti-PD-1 antibodies) and atezolizumab and durvalumab (anti-PD-L1 antibodies), block this interaction and thereby restore the ability of cytotoxic T-cells to recognize and destroy cancer cells [4]. Although these therapies have significantly improved survival in some patients, the overall response rates remain limited, with only approximately 14-26% of patients deriving durable clinical benefit from PD-1/PD-L1 therapy [8, 9]. These treatments are also associated with substantial economic burden. In the Netherlands, pembrolizumab, one of the most widely used PD-1 inhibitors, has a list price of approximately €2,624 per flacon, and standard dosing over 12 months can approach €90,000 per patient per year depending on treatment duration and survival outcomes [10].

From a clinical standpoint, immune checkpoint inhibitors are generally better tolerated than cytotoxic chemotherapy but nevertheless cause frequent adverse events. Approximately 60-80% of patients experience at least one treatment-related adverse event, most commonly fatigue, rash, diarrhea, nausea, or musculoskeletal symptoms [11]. Furthermore, 10-20% of patients develop moderate to severe immune-related toxicities such as colitis, hepatitis, endocrinopathies, and pneumonitis, which may require immunosuppressive therapy or treatment discontinuation [11, 12].

Current research focuses on several categories of biomarkers that may influence immunotherapy response in NSCLC. The most studied biomarkers include PD-L1 expression, which reflects the level of target protein on tumor cells [13]; Tumor Mutational Burden (TMB), which represents the number of mutations that can create neoantigens [14]; Microsatellite Instability (MSI), which indicates defects in DNA mismatch repair leading to increased immunogenicity [15]; and circulating biomarkers, such as cell-free DNA (cfDNA) and cytokine profiles, which provide non-invasive insight into

tumor dynamics and immune activity [16]. The aim of this literature review is to evaluate the predictive value of these biomarkers and to assess to what extent they can predict response to PD-1/PD-L1 checkpoint inhibitor therapy in patients with NSCLC.

3 Results

3.1 PD-L1

PD-L1 expression on tumor cells and, to a lesser extent, on tumor-infiltrating immune cells is considered a predictive biomarker because it reflects activation of the PD-1/PD-L1 immune checkpoint pathway directly targeted by immune checkpoint inhibitors. Biologically, PD-L1 is a membrane-bound ligand expressed on tumor cells and antigen-presenting cells that binds to the PD-1 receptor on activated T cells, transmitting an inhibitory signal that suppresses T-cell proliferation and cytokine production and thereby facilitates tumor immune evasion [17]. Because PD-1/PD-L1 inhibitors act by blocking this interaction, PD-L1 expression represents a biologically plausible surrogate for target engagement and has been associated with improved clinical outcomes in NSCLC across multiple studies [17].

In clinical practice, PD-L1 expression is assessed by immunohistochemistry (IHC) on formalin-fixed paraffin-embedded (FFPE) tumor tissue, most commonly obtained from diagnostic tumor biopsies or resection specimens. Validated assays include Dako 22C3, Dako 28-8, Ventana SP142, and Ventana SP263, each developed as a companion or complementary diagnostic for specific checkpoint inhibitors. These assays differ in antibody clone, staining platform, antigen retrieval chemistry, and scoring methodology, which contributes to variability in PD-L1 classification [18]. Notably, the SP142 assay consistently stains fewer tumor cells than 22C3 or SP263, resulting in discordant PD-L1 status in up to 30% of cases [18]. PD-L1 expression is commonly reported as the Tumor Proportion Score (TPS), defined as the percentage of viable tumor cells with membranous staining, or as the Combined Positive Score (CPS), which additionally includes PD-L1-positive immune cells [19].

High PD-L1 expression correlates with improved clinical outcomes. In the KEYNOTE-024 trial, patients with TPS $\geq$ 50% treated with pembrolizumab showed significantly longer progression-free survival (10.3 vs. 6.0 months) and overall survival compared with chemotherapy [20]. Similarly, KEYNOTE-042 demonstrated an overall survival benefit at TPS $\geq$ 1% [21]. However, the clinical utility of PD-L1 is limited by inter-assay variability, imperfect reproducibility, and biological heterogeneity [22, 18].

PD-L1 expression varies spatially within tumors and between primary and metastatic lesions, and it is dynamically regulated over time. Moreover, PD-L1 expression can be upregulated or downregulated following chemotherapy, radiotherapy, or targeted therapy, largely through inflammation- and interferon-γ-mediated signaling pathways [23]. As a result, PD-L1 assessment from a single pretreatment biopsy may not accurately reflect the tumor immune status at the time of immunotherapy initiation. While this dynamic regulation limits the reliability of PD-L1 as a static predictive biomarker, it also suggests that prior or concurrent treatments such as radiotherapy may modulate the tumor microenvironment in ways that could enhance responsiveness to immune

checkpoint inhibition, although such effects are highly context-dependent.

These biological and technical limitations restrict the predictive accuracy of PD-L1 testing. In KEYNOTE-024, the objective response rate (ORR) was 44.8% in patients with TPS $\geq$ 50%, compared with 27% in TPS 1-49% and approximately 10-15% in PD-L1-negative tumors, indicating that more than half of PD-L1–high patients do not respond to treatment [20].

Meta-analyses further demonstrate the limited discriminative ability of PD-L1. A systematic review of 22 studies reported a pooled sensitivity of approximately 64% and specificity of approximately 49% for PD-L1 positivity in predicting response to PD-1/PD-L1 inhibitors in NSCLC, a performance that approaches random classification rather than reliable discrimination between responders and non-responders [24]. Importantly, 8-20% of PD-L1-negative patients still respond to immunotherapy, while many PD-L1-high patients fail to respond. Together, these findings indicate that PD-L1 enriches for responders but cannot reliably distinguish responders from non-responders [14].

Given these limitations, one potential strategy that has been proposed is to evaluate PD-L1 expression in combination with additional biomarkers, rather than relying on PD-L1 as a standalone predictor [25].

3.2 Tumor Mutational Burden

Tumor Mutational Burden (TMB) is considered a promising biomarker because tumors with a high number of somatic mutations can be expected to generate more neoantigens, increasing their visibility to the immune system and potentially enhancing responsiveness to immune checkpoint inhibitors [14].

TMB is defined as the number of somatic mutations per megabase (mut/Mb) of tumor DNA. Evidence for its predictive value in NSCLC emerged from the CheckMate 227 trial, in which patients with TMB $\geq$ 10 mut/Mb treated with nivolumab-ipilimumab achieved a median progression-free survival of 7.2 months compared with 5.5 months in the chemotherapy group, representing an improvement of approximately 1.7 months [25]. However, TMB has shown inconsistent predictive performance across studies, particularly in PD-1/PD-L1 monotherapy, where some analyses report minimal or no correlation with survival outcomes [26].

Several factors may explain these discrepant results. First, not all somatic mutations generate immunogenic neoantigens capable of being presented on major histocompatibility complex molecules. Second, transcriptomic and immune-profiling studies demonstrate that some tumors with high TMB lack interferon-γ signaling and cytotoxic T-cell infiltration, indicating that high mutational burden alone does not guarantee an effective antitumor immune response [14]. This may reflect additional mechanisms of immune escape, such as defective antigen presentation, exclusion of T cells from the tumor microenvironment, or the presence of immunosuppressive cell populations that prevent effective immune activation despite a high neoantigen load. Third, differences in treatment regimens, especially combination immunotherapy versus monotherapy, may influence the relevance of TMB as a predictive biomarker.

Practical limitations further reduce the clinical utility of Tumor Mutational Burden. Differences in sequencing depth, genomic panel size, and bioinformatic pipelines result

in poor standardization across institutions. In addition, high TMB is significantly more common in smokers due to tobacco-associated mutational signatures [27, 28]. While smoking history is therefore strongly correlated with elevated TMB, several studies have reported improved immunotherapy outcomes in patients with a smoking history that are not fully explained by TMB alone [29, 25, 30]. This suggests that smoking-associated tumors may harbor qualitative differences in neoantigen composition, clonal architecture, or immune microenvironment that enhance immunogenicity beyond what is captured by a simple mutation count. These observations further underscore that TMB reflects only one aspect of tumor immunogenicity and must be interpreted within a broader biological context.

Overall, TMB provides important biological insight but lacks sufficient reliability, standardization, and biological specificity to function as a standalone predictive biomarker.

3.3 Microsatellite Instability

Microsatellite Instability (MSI) arises from defects in the DNA mismatch repair (MMR) system, which normally corrects base-base mismatches and small insertion-deletion loops generated during DNA replication. Deficient MMR is most commonly caused by loss-of-function mutations or epigenetic silencing of the core MMR genes MLH1, MSH2, MSH6, and PMS2. Microsatellites are short, repetitive DNA sequences that are particularly prone to replication errors; failure to repair insertion-deletion mutations in these regions leads to frameshift mutations, altered C-terminal protein sequences, and truncated proteins. As a consequence, MSI-high (MSI-H) tumors generate a large number of frameshift-derived neoantigens that are highly immunogenic, providing a strong biological rationale for their pronounced sensitivity to immune checkpoint inhibition [31].

MSI-high (MSI-H) status is defined using PCR-based microsatellite panels or next-generation sequencing, based on instability in a predefined number of microsatellite loci. Clinical trials have demonstrated that MSI-H tumors are strongly associated with response to PD-1 blockade, with objective response rates of approximately 40-50% in mismatch repair–deficient colorectal and endometrial cancers, compared with minimal responses in microsatellite-stable tumors [31].

In NSCLC, microsatellite instability–high (MSI-H) tumors are extremely rare, large-scale genomic profiling studies consistently estimate the true frequency to be approximately 0.3–0.5% [32, 33, 34]. Consequently, evidence in lung cancer is limited to very small cohorts and individual case reports. In the largest available analyses of mismatch repair–deficient solid tumors treated with PD-1 blockade, pembrolizumab achieved objective response rates of approximately 53%, with complete responses observed in about 21% of patients across diverse tumor types, consistent with the 40–50% response rates reported in MSI-H cancers [31]. However, the very small number of MSI-H NSCLC cases precludes reliable estimation of response rates specific to lung cancer and limits broad generalization. Nevertheless, these findings strongly support the biological plausibility of MSI as a predictive biomarker in NSCLC despite its very low prevalence.

Although MSI testing is not routinely performed in NSCLC, large-scale genomic

profiling studies show that MSI-H/MMR-D occurs in only approximately 0.3–0.5% of NSCLC cases, consistent with true biological rarity rather than underdetection alone. In cohorts of over 5,000, 1,500, and 12,000 lung cancer patients profiled by next-generation sequencing, MSI-H was identified in 0.41%, 0.39%, and 0.5% of cases, respectively, confirming its low prevalence in this tumor type [32, 33, 34].

Quantitatively, MSI-H demonstrates substantially stronger predictive performance than PD-L1 expression or Tumor Mutational Burden. Across tumor types, MSI-H is associated with objective response rates of approximately 40–50% to PD-1 blockade, whereas PD-L1 expression in NSCLC yields response rates of 45% even in the highest expression group (TPS $\geq$ 50%), with pooled sensitivity and specificity close to random classification. TMB showed more modest and context-dependent effects, with progression-free survival improvements of approximately 1–2 months in selected treatment settings and inconsistent associations with response in monotherapy. These differences highlight that MSI-H represents a high-impact but rare biomarker for ICI therapy prediction in NSCLC, whereas PD-L1 and TMB are more broadly applicable but individually less predictive.

3.4 Circulating Biomarkers

Circulating biomarkers provide a minimally invasive approach to monitoring tumor burden and immune activity over time, without requiring repeated tumor biopsies. Cell-free DNA (cfDNA) and circulating tumor DNA (ctDNA) are therefore best described as potential circulating biomarkers rather than established predictors of immunotherapy response.

High baseline levels of circulating cell-free DNA (cfDNA), typically defined as values in the upper quartile of the study population, are consistently associated with poor prognosis in NSCLC, with higher plasma cfDNA concentrations linked to shorter progression-free and overall survival across multiple cohorts and pooled analyses [35, 36, 37], reflecting increased overall tumor burden. More importantly, longitudinal analyses demonstrate that early on-treatment changes in circulating tumor DNA (ctDNA) are strongly associated with clinical outcomes during PD-1/PD-L1 therapy. Specifically, declines in ctDNA detected within the first weeks of treatment often occur several weeks before corresponding changes are visible on radiographic imaging, such as tumor shrinkage or radiographic progression, typically preceding imaging-based assessment by approximately 4–8 weeks [38, 39]. These early ctDNA dynamics are thought to reflect effective tumor cell killing or emerging resistance at a molecular level, resulting in reduced or increased release of tumor-derived DNA into the circulation.

Cytokines have also been investigated as systemic biomarkers of immunotherapy response. In particular, elevated baseline serum interleukin-8 (IL-8) levels have been consistently associated with poor outcomes during PD-1/PD-L1 therapy across multiple cancer types, including NSCLC [40]. In large multicohort analyses, patients with high IL-8 levels showed significantly shorter progression-free and overall survival, with hazard ratios of approximately 2-3 compared with patients with low IL-8 levels. Moreover, dynamic changes in IL-8 during treatment correlated with clinical outcomes, with decreasing IL-8 levels associated with tumor regression and increasing levels associated with disease progression [40].

Biologically, IL-8 is produced by tumor cells and cells of the tumor microenvironment, including macrophages and neutrophils, and plays a central role in recruiting neutrophils and myeloid-derived suppressor cells. Elevated IL-8 levels therefore reflect a tumor microenvironment dominated by myeloid-driven inflammation and immune suppression, which can inhibit effective cytotoxic T-cell activity and limit the efficacy of immune checkpoint inhibition. In contrast, interferon-γ-related gene expression signatures in tumor tissue reflect an inflamed, T-cell-infiltrated tumor microenvironment and have been associated with improved response rates to PD-1 blockade [14, 17].

Other circulating immune-related markers, including soluble PD-L1, circulating CD8+ T cells, and myeloid-derived suppressor cells, have been investigated primarily in exploratory and correlative clinical studies rather than as validated predictive biomarkers. These markers have been assessed in small prospective cohorts and retrospective analyses of patients receiving PD-1/PD-L1 inhibitors, where associations with treatment outcomes have been reported but not consistently reproduced [16]. Soluble PD-L1 represents a circulating form of the PD-L1 protein and has been proposed to contribute to systemic immune suppression, while circulating immune cell subsets reflect the balance between immune activation and immune inhibition within the host immune system.

Although these biomarkers are supported by biological plausibility and preliminary clinical associations, they have not been validated for routine clinical decision-making. Their interpretation is limited by substantial assay variability, biological confounding factors such as infection or inflammation, and the absence of standardized methodologies or clinically validated cutoff values [16]. As a result, they currently remain exploratory and are best considered complementary research tools rather than actionable predictors of immunotherapy response.

Nevertheless, circulating biomarkers represent one of the most rapidly evolving areas of NSCLC immunotherapy research, particularly as adjuncts to tissue-based biomarkers and as tools for longitudinal monitoring of treatment response and resistance.

4 Discussion

Several biomarkers have been investigated for their ability to predict response to PD-1/PD-L1 immune checkpoint inhibitor therapy in NSCLC, including PD-L1 expression, Tumor Mutational Burden, Microsatellite Instability, and circulating biomarkers such as circulating tumor DNA. While each of these biomarkers is associated with immunotherapy outcomes, none provides sufficient predictive accuracy to guide treatment exclusion. Importantly, the central clinical challenge is not identifying patients who will definitively respond to immunotherapy, but rather identifying those who are highly unlikely to benefit and in whom treatment could be safely withheld. As discussed below, each biomarker captures a distinct aspect of tumor–immune biology; however, biological heterogeneity, technical variability, and limited negative predictive value prevent any of them from functioning as a reliable standalone predictor [4, 14].

PD-L1 expression remains the most established biomarker in routine clinical practice and reflects the presence of the therapeutic target of PD-1/PD-L1 inhibitors. Clinical trials consistently demonstrate improved outcomes in patients with high PD-L1

expression, particularly at tumor proportion scores (TPS) $\geq$ 50% [20, 21]. However, substantial response heterogeneity persists: many PD-L1–high patients fail to respond, while a meaningful proportion of PD-L1–negative patients derive clinical benefit [20, 14]. In addition, assay-related variability, intratumoral heterogeneity, and dynamic regulation of PD-L1 expression limit its reproducibility and predictive accuracy [22, 23, 41]. Meta-analyses further demonstrate that the discriminatory ability of PD-L1 approaches random classification, emphasizing that PD-L1 functions as an enrichment marker rather than a reliable tool for identifying non-responders [24].

As summarized above, Tumor Mutational Burden provides a biologically plausible but incomplete measure of tumor immunogenicity. However, its inconsistent predictive performance highlights key conceptual and methodological limitations. TMB quantifies the number of somatic mutations rather than the immunogenic quality of the resulting neoantigens; many mutations do not generate peptides that are effectively processed, presented on major histocompatibility complex molecules, or recognized by T cells [14, 42]. Consequently, high TMB does not necessarily translate into effective antitumor immune activation. Moreover, TMB does not account for downstream processes essential for immune checkpoint inhibitor efficacy, including intact antigen presentation, interferon-γ signaling, and cytotoxic T-cell infiltration. Tumors with high mutational burden but an immunologically "cold" or immune-excluded microenvironment may therefore fail to respond despite abundant potential neoantigens [14, 26]. This biological disconnect explains why TMB captures only one step of a multistep immune response and why its predictive value is not consistently superior to PD-L1 expression, particularly in PD-1/PD-L1 monotherapy settings [14, 21, 24, 41, 14, 26]. Methodological variability further limits the clinical reliability of TMB. Differences in sequencing panel size, mutation calling pipelines, and cutoff definitions introduce substantial heterogeneity across studies [26, 27]. Larger gene panels and whole-exome sequencing provide more stable estimates than small targeted panels, but no single gene set or cutoff has been universally accepted [27, 28]. Although treating TMB as a continuous variable may better reflect underlying biology, this approach has limited clinical utility because it does not yield clear decision thresholds for treatment exclusion. Importantly, TMB is strongly confounded by smoking status, as tobacco exposure generates characteristic mutational signatures that inflate mutational burden [27, 28]. While smokers often demonstrate improved immunotherapy outcomes, this association is unlikely to be explained by mutation count alone. Smoking-related tumors tend to harbor clonal, truncal neoantigens and exhibit distinct immune microenvironment features that enhance immunogenicity beyond what is captured by TMB alone [25, 29, 30]. These observations further support the interpretation of TMB as a contextual biomarker rather than a standalone predictor capable of identifying definite non-responders.

Microsatellite Instability represents a contrasting example of a biologically strong but clinically rare biomarker in NSCLC. MSI-high tumors exhibit mismatch repair deficiency and extreme genomic instability, leading to abundant frameshift-derived neoantigens and robust immune recognition. Across tumor types, MSI-high status is strongly associated with response to PD-1 blockade [31]. In NSCLC, however, MSI-high tumors are exceedingly rare, occurring in approximately 0.3–0.5% of cases [32, 33, 34]. While screening can reliably identify this small subgroup of exceptional responders, the overwhelming majority of patients without mismatch repair deficiency

would still receive immune checkpoint inhibitor therapy. Thus, MSI primarily identifies patients who are highly likely to benefit, rather than those who can be confidently excluded from treatment.

Circulating biomarkers offer a complementary perspective by providing dynamic, minimally invasive insights into tumor burden and immune response. In particular, changes in circulating tumor DNA during treatment have been consistently associated with clinical benefit and often precede radiographic response or progression by several weeks [38, 39]. These findings indicate that ctDNA functions primarily as a marker of real-time treatment efficacy rather than baseline sensitivity to immunotherapy. However, no circulating biomarker has yet demonstrated sufficient validation, standardization, or predictive accuracy to support treatment exclusion decisions, and current evidence remains largely observational [35, 36, 37]. Beyond ctDNA, accumulating evidence suggests that biomarkers capturing the functional immune state of the tumor microenvironment may provide greater predictive insight. Tumor immune contexture, including CD8+ T-cell infiltration and interferon-γ–related gene expression signatures, reflects pre-existing immune engagement and has been consistently associated with response to immune checkpoint inhibition [14, 17]. Conversely, defects in antigen presentation machinery, such as loss of HLA class I expression or β2-microglobulin deficiency, as well as systemic immunosuppressive markers including elevated interleukin-8 levels, neutrophil-to-lymphocyte ratio, and myeloid-derived suppressor cell populations, have been implicated in primary resistance and poor clinical outcomes [16, 40]. Taken together, these findings indicate that the limited predictive performance of individual biomarkers reflects not only technical shortcomings but also fundamental biological complexity. Each biomarker captures a distinct yet incomplete aspect of the tumor–immune interaction, and none provides sufficient negative predictive value to justify withholding immune checkpoint inhibitor therapy from biomarker-negative patients. Future advances in patient selection are therefore likely to depend on integrated biomarker strategies that combine tumor immune contexture, antigen presentation capacity, and dynamic response markers rather than reliance on PD-L1 expression, Tumor Mutational Burden, or circulating biomarkers alone. Prospective studies incorporating standardized assays, correction for key confounders such as smoking and immune activation, and longitudinal sampling will be essential. Such studies are required to validate multidimensional biomarker approaches and translate them into clinically actionable tools.

5 Conclusion

This literature review demonstrates that multiple biomarkers are associated with response to PD-1/PD-L1 checkpoint inhibitors in non-small cell lung cancer, but that none provide sufficient predictive accuracy when used alone. PD-L1 expression remains the most widely implemented biomarker in clinical practice, yet its predictive value is limited by biological heterogeneity, assay variability, and modest discriminatory performance. Tumor Mutational Burden and Microsatellite Instability offer strong biological rationale but are constrained by lack of standardization and low prevalence, respectively. Circulating biomarkers, particularly ctDNA dynamics, show promise for

early response assessment but are not yet validated for routine clinical use. Importantly, the current evidence indicates that none of the available biomarkers provides adequate certainty to identify patients who will definitively not benefit from immune checkpoint inhibitor therapy, and thus cannot be used to justify treatment exclusion. Overall, immunotherapy response in NSCLC cannot be reliably predicted by a single biomarker. Future advances are likely to depend on integrated biomarker strategies that combine baseline tumor characteristics with dynamic measures of treatment response. Prospective studies will be required to validate such approaches and to translate them into clinically actionable tools that improve patient selection and treatment outcomes.

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