ImmunoTargets and Therapy

Dovepress
Taylor & Francis Group

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REVIEW

Emerging Predictive Biomarkers of Immunotherapy Sensitivity in Patients with Non-Small Cell Lung Cancer

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Abstract: In recent years, the therapeutic landscape of non-small cell lung cancer (NSCLC) has been transformed by immune checkpoint inhibitors (ICIs), which have led - in some patients - to unprecedented survival expectancy. Nevertheless, identifying patients most likely to benefit from ICI remains a major challenge. While PD-L1 expression and tumor mutation burden (TMB) represent established predictive biomarkers, their predictive ability still needs to be improved, which underscores the need for identifying additional (bio)markers for treatment selection. Recent research has highlighted multiple emerging biomarkers, including genomic alterations (eg, *KEAP1, STK11, SMARCA4*), markers of metabolic pathway dysregulation (IDO, adenosine axis), tumor-infiltrating lymphocytes, and blood-based biomarkers (eg soluble markers of inflammation, germline HLA diversity, and circulating tumor DNA). Host-related determinants, such as the history of tobacco exposure and the body mass index, further contribute to immunotherapy outcomes. In addition, artificial intelligence (AI) and machine learning (ML) approaches are enabling integration of multidimensional data, leading to predictive scoring systems which have outperformed conventional biomarkers in certain settings. This review synthesizes current evidence on established and emerging predictive biomarkers in NSCLC, highlighting the potential of combining biological, host, and computational features to inform precision immunotherapy strategies.

Keywords: NSCLC, immunotherapy, predictive biomarkers, tumor microenvironment, host factors, artificial intelligence

Introduction

In recent years, a deeper understanding of the molecular mechanisms driving carcinogenesis and immune escape has led to the development of novel therapeutic agents, resulting in unprecedented survival outcomes for patients with non-small cell lung cancer (NSCLC).[1] The current therapeutic landscape relies on two principal strategies: targeted therapies for patients harboring actionable genomic alterations (AGAs) and immune checkpoint inhibitors (ICIs), with or without chemotherapy, for those without AGAs.[2] ICIs mediate tumor cells killing by reactivating T cells and restoring the antitumor immune response. In particular, ICIs currently used in clinical practice are monoclonal antibodies that disrupt immune checkpoints (eg, PD-1/PD-L1 and CTLA-4/CD80-86), thereby enhancing the host immune response against cancer. Although many patients derive significant clinical benefit from immunotherapy, virtually all eventually develop resistance, either primary or acquired. While multiple therapeutic strategies are being explored to overcome this challenge, the identification of robust predictive biomarkers to inform treatment selection remains a critical unmet need.

https://doi.org/10.2147/ITT.S567238
Received: 16 September 2025
Accepted: 24 January 2026
Published: 2 February 2026

This review introduces established biomarkers used to predict ICI efficacy in NSCLC - namely programmed death-ligand 1 (PD-L1) expression and tumor mutation burden (TMB) – and explores other emerging, promising (bio)markers. To improve clarity, we grouped the emerging predictors of immunotherapy sensitivity discussed in this review into three broad, non-mutually-exclusive categories:

- Tissue-based biomarkers, which rely on adequate tumor sampling and include genomic alterations, tumor microenvironment (TME) characteristics, markers of immunosuppressive metabolic pathways, alternative immune checkpoints, and tumor HLA class I diversity evaluated from tissue;
- Soluble biomarkers, which represent minimally invasive tools and have the potential to overcome the limitations of repeated biopsies. These include inflammatory markers, liquid biopsy—especially circulating tumor DNA (ctDNA)—and germline-derived HLA diversity assessed through peripheral blood;
- Host-related factors, such as tobacco use history, sex, and body mass index (BMI), which have shown associations with ICI outcomes.

Finally, we will discuss how artificial intelligence (AI) may contribute to integrating multiple (bio)markers types and play a role in shaping personalized treatment strategies for NSCLC.

Established Predictors of Immunotherapy Response: PD-L1 and TMB

At present, two biomarkers — PD-L1 and TMB — represent the cornerstone for treatment decision-making in NSCLC without AGAs.[3] Both biomarkers have been extensively studied so far, and are widely accepted for informed clinical decision regarding sensitivity to ICI-based therapies.

Importantly, PD-L1 expression early emerged as a key predictive biomarker for ICI-based therapy in NSCLC.[4] Assessment of the levels of PD-L1 expression on tumor tissue is performed by immunohistochemistry (IHC) using assays like Dako 22C3 and Ventana SP263 or SP142, with results reported as the percentage of cancer cells with membrane staining of any intensity (tumor proportion score, TPS), or, specifically for SP142, as the percentage of positive PD-L1 expression either on tumor cells or tumor-infiltrating immune cells.[4,5]

For the selection of patients more likely to benefit from anti–PD-(L)1 monotherapy, a PD-L1 TPS ≥50% has emerged as the most reliable threshold. The pivotal KEYNOTE-024, IMpower110, and EMPOWER-Lung 1 trials demonstrated superior survival of pembrolizumab, atezolizumab, and cemiplimab, respectively, compared with platinum-based chemotherapy in untreated advanced NSCLC with PD-L1 TPS ≥50%,[6–8] with 5-year survival reaching approximately 30% with pembrolizumab and cemiplimab versus 15% with chemotherapy.[9,10] Furthermore, data from a large real-world cohort indicated that the addition of chemotherapy to anti–PD-(L)1 therapy in this population did not improve progression-free survival (PFS) or overall survival (OS), supporting the preferential use of ICI monotherapy in patients with PD-L1 TPS ≥50%.[11] Conversely, the addition of chemotherapy to immunotherapy remains the standard approach for patients with PD-L1 <50%.[3]

Despite its predictive value, PD-L1 is an imperfect biomarker for several reasons, which are summarized in Table 1. First, PD-L1 expression is quantitative, meaning that it should be interpreted as a continuous variable. Retrospective analyses indicate that, even within the broad PD-L1 categories of ≥50% and <50%, finer thresholds can identify subgroups with different sensitivity to ICI-based therapies.[12] Relying solely on the 50% threshold may limit treatment personalization, potentially overlooking patients who derive minimal benefit from immunotherapy (eg, PD-L1<1%) and who might be candidates for treatment escalation, such as the addition of a CTLA-4 inhibitor.[13,14]

Second, PD-L1 expression has spatial and temporal heterogeneity, as it may be significantly influenced based on the site that has been biopsied and on the moment of its measurement.[15]

Finally, inter-assay and inter-observer variability may further complicate its reliability and reproducibility.[16,17]

The limitations of PD-L1 do not eliminate its central role, rather underscore the urgent need to integrate it with additional biomarkers.

TMB represents the total number of somatic non-synonimous mutations per megabase (mut/Mb) in the cancer genome. The underlying rationale for using TMB as a predictive biomarker for immunotherapy is that a higher

Table 1 Advantages and Disadvantages of PD-L1 and TMB Biomarkers

PD-L1	
ADVANTAGES	**DISADVANTAGES**
Truly predictive: while its predictive role has been confirmed, its prognostic role is uncertain	**Quantitative**: PD-L1 expression is a biological continuum
Consistent: virtually all ICI-based therapies in NSCLC have shown a correlation between clinical benefit and levels of PD-L1 expression	**Dynamic**: spatial and temporal heterogeneity are the background
Easily available: IHC provides readiness and reproducibility with different clones	**Restricted**: it does not take into account the tumor microenvironment
Applicable to different samples: also possible on FNA and cell blocks provided that at least 100 viable tumor cells are present	**Concordancy**: Inter-assay, -laboratory and -observer variability may influence the results
TMB	
ADVANTAGES	**DISADVANTAGES**
Truly predictive: high TMB (≥10 mut/Mb) identifies patients more likely to to benefit from ICI-based therapies, particularly dual ICI, independently of PD-L1 expression	**Lack of standardization**: no universally accepted assay or cut-off; results vary across platforms (WES ideal, but impractical)
Quantitative: TMB is a continuous variable, with higher levels associated to superior ICI outcomes	**Concordancy**: inter-assay and inter-laboratory variability; requires harmonization (eg, z-score) for comparability across multiple institutions
Biologically consistent: reflects tumor neoantigen burden and enhanced tumor immunogenicity	**Technically restricted**: higher cost, longer turnaround time and limited availability compared with IHC
Complementary: refines patient stratification within PD-L1 subgroups	**Dynamic**: spatial and temporal heterogeneity may influence TMB assessment
Applicable to different samples: feasible on tissue, using multiple NGS panels, or blood (bTMB)	**Uncertain prognostic role**: primarily predictive rather than prognostic, requiring integration with other biomarkers

Abbreviations: PD-L1, programmed death-ligand 1; ICI, immune checkpoint inhibitor; NSCLC, non-small cell lung cancer; IHC, immunohistochemistry; FNA, fine-needle aspiration; TMB, tumor mutational burden; NGS, next-generation sequencing; WES, whole-exome sequencing; mut/Mb, mutations per megabase; bTMB, blood tumor mutational burden.

mutational load increases the likelihood of generating neoantigens, which may, in turn, render tumors more immunogenic and susceptible to immune attack.

Initially, a cut-off of 10 mut/Mb as assessed by FoundationOne CDx was set in order to select patients who could benefit from the use of dual anti-PD-1/CTLA-4 blockade with nivolumab plus ipilimumab[18] Subsequently the CheckMate 227 trial confirmed that high TMB patients (≥10 mut/Mb) derived greater benefit from dual ICI blockade regardless of PD-L1 expression, thus suggesting that PD-L1 and TMB may act as different, though complementary, biomarkers of benefit from ICI-based therapies.[19]

Nevertheless, despite these promising findings, the adoption of TMB in routine practice has proven difficult (Table 1). That is mainly due to the lack of universally accepted assays or cut-off values. Although whole-exome sequencing (WES) technique offers the most comprehensive evaluation, it is not feasible for widespread clinical use. Therefore, more practical targeted next-generation sequencing (NGS) panels have become available (eg FoundationOne CDx), but owing to their cost and variable results, comparisons across studies and Institutions are slightly complex.[20] To bypass the above-mentioned issue, standardization methods have been proposed. Vokes et al used standardization to z-score for comparing TMB distribution across different platforms. In doing so, they were able to identify a different cut-off of sensitivity to ICI-based therapies in terms of mut/Mb for each of the platform that was taken into account.[21] By using the same methodology, they were also able to hypothesize that patients with TMB greater or equal to the 90th percentile were those with the utmost benefit from treatment with ICI-based regimens.[22,23]

To conclude, the available data underscore the complexity of immune responsiveness in relation to TMB levels, and support the integration of both TMB and PD-L1 into multifactorial or composite (bio)markers. Such models are currently under investigation and may provide a more nuanced prediction of treatment outcomes in the near future.[24]

Emerging Tissue Biomarkers: From Research to Clinical Application

Beyond PD-L1 and TMB, several techniques are either being employed or under investigation to further characterize NSCLC and predict its sensitivity to different ICI-based regimens, many of which still rely on the availability of tumor tissue obtained through histological sampling. Tissue-based biomarkers are depicted in Figure 1.

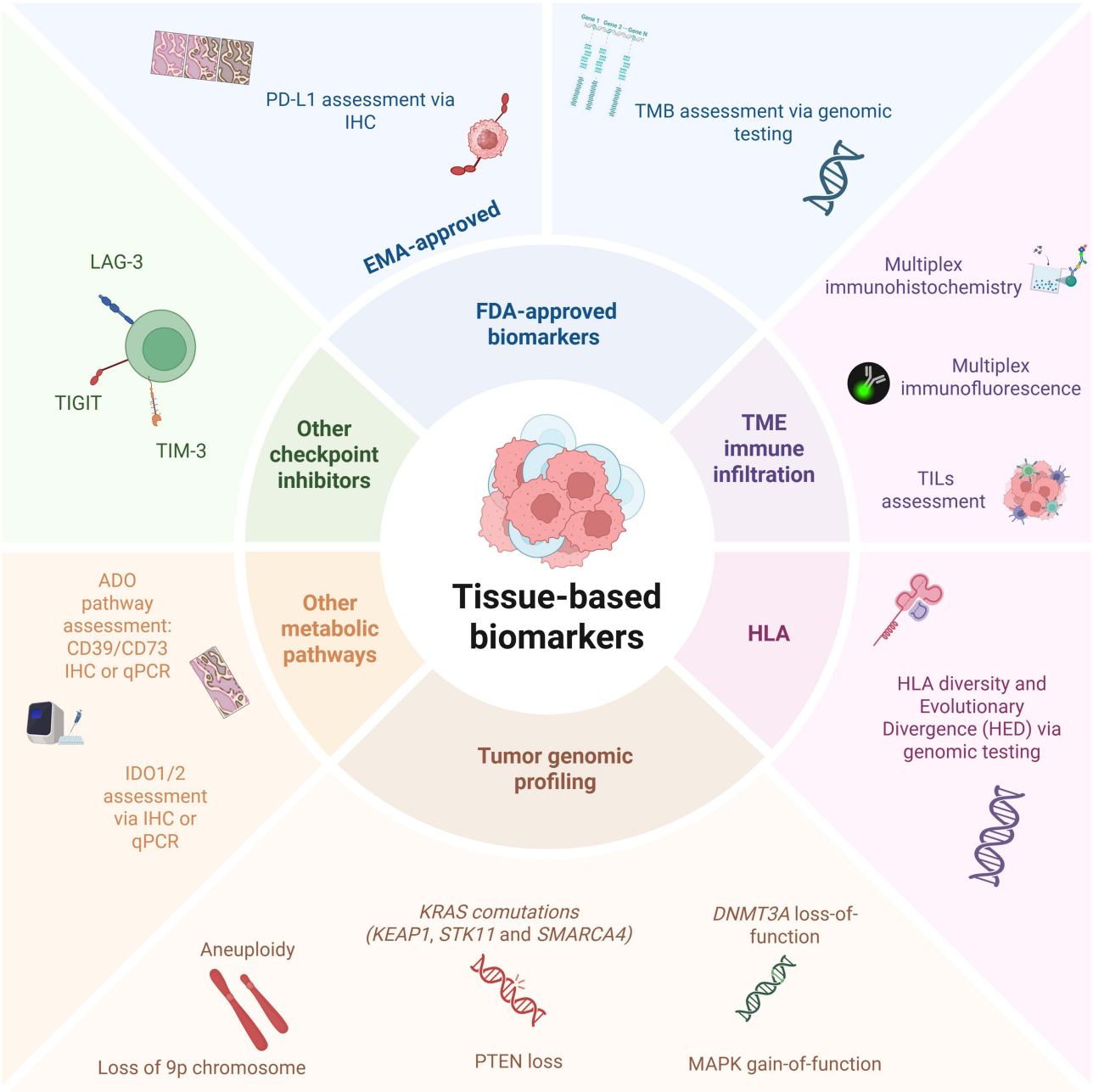

Figure 1 Tissue-based biomarkers for immune checkpoint inhibitor response in non–small cell lung cancer. Overview of regulatory-approved and emerging tissue-derived biomarkers, including PD-L1 expression, tumor mutational burden, tumor immune microenvironment features, HLA class I diversity, and tumor genomic alterations.

https://doi.org/10.2147/ITT.S567238

Genomic Profiling

Routine comprehensive tumor genomic profiling has paved the way for the inclusion of multiple genomic factors – beyond AGAs - into the therapeutic stratification of NSCLC. Among these, mutations in *KEAP1* and *STK11*, now recognized as robust biomarkers of resistance to PD-(L)1 blockade, are increasingly used to refine prognostic assessment in NSCLC.[23,25–27] Interestingly, loss-of-function mutations in these genes appear to adversely affect immunotherapy outcomes especially in the context of a concurrent *KRAS* mutation. Tumors harboring *KRAS/STK11* or *KRAS/KEAP1* co-mutations exhibit distinctive immune landscapes, characterized by unique gene expression signatures and altered patterns of immune cell infiltration.[26] In particular, transcriptomic analyses revealed a marked downregulation of key immune pathways—including MHC class II complex, T-cell activation, immune response signaling, leukocyte migration and degranulation, and myeloid cell activation—in patients harboring concurrent *KRAS* and *STK11/KEAP1* mutations, even when compared with *KRAS*-mutant/*STK11-KEAP1* wild-type NSCLC. These co-mutant tumors also displayed reduced infiltration of CD8+ T cells and B cells. Notably, Skoulidis et al demonstrated that the addition of CTLA-4 inhibition can overcome *KEAP1/STK11*-associated resistance to PD-(L)1 blockade, offering a potential strategy to improve outcomes in this challenging patient population.[28]

Beyond *KEAP1* and *STK11*, other genomic alterations can also shape the immune landscape of *KRAS*-mutant NSCLC. One notable example is the SWItch/Sucrose Nonfermentable (SWI/SNF) chromatin-remodeling complex, whose disruption has been linked to unfavorable immunotherapy outcomes. Within this family of genes, *SMARCA4* has emerged as the most impactful determinant of ICI resistance, with loss-of-function mutations consistently associated with diminished therapeutic benefit.[29]

The network of genomic determinants influencing ICI response further extends to tumor suppressors involved in signaling regulation. In this regard, *PTEN* plays a pivotal role: loss-of-function mutations in this gene dysregulate the PI3K/AKT signaling cascade and promote infiltration by regulatory T cells (Tregs), thereby impairing tumor sensitivity to immune checkpoint inhibition.[30] In contrast, activating mutations in the MAPK pathway have been associated with increased sensitivity to dual ICI therapy in squamous NSCLC, highlighting the importance of broad genomic profiling even in this histology, where NGS is often underutilized.[31]

Another gene gaining increasing interest in predicting ICI outcomes is *DNMT3A*. Somatic loss-of-function mutations in *DNMT3A* are found across several cancer types, including up to 4–5% of NSCLC, and are associated with a distinct immunophenotype characterized by upregulated PD-1 and IFNγ signatures and an immune-enriched TME.[32–35] Ricciuti et al demonstrated that *DNMT3A* mutations are significantly enriched among responders to immunotherapy, correlating with improved objective response rates as well as longer PFS and OS.[35] These findings highlight *DNMT3A* role not only as a predictive biomarker but also as a potential therapeutic target. Therefore, strategies aimed at modulating the activity or expression of *DNMT3A* may enhance NSCLC immunogenicity and improve ICI efficacy.

In addition to mutational events, copy number alterations (CNAs), defined as gains or losses of large chromosomal segments, have emerged as significant prognostic biomarkers. Several retrospective studies have demonstrated that high levels of CNAs correlate with poor outcomes to ICI in solid tumors, including NSCLC.[36,37] Given that aneuploidy represents an extensive type of CNA, affecting larger regions of the cancer genome, its distinct prognostic significance in NSCLC has been the subject of further investigation. Retrospective analyses have shown that aneuploidy serves as an independent, robust biomarkers and is associated with immunosuppressive TME and reduced ICI efficacy in both locally advanced and metastatic NSCLC.[38,39] A genomic region of particular interest is chromosome 9p. Han et al reported that homozygous deletion of 9p21 is associated with poor outcomes under anti–PD-L1 monotherapy, independently of PD-L1 expression and TMB. Notably, approximately half of 9p21.3 deletions also involve the type I interferon (IFN) gene cluster, thus suggesting a role for impaired IFN signaling in resistance to ICIs.[40] Consistently, Ebot et al demonstrated that, among non-squamous NSCLC patients treated with single-agent immunotherapy, tumors harboring 9p21.3 deletions - intriguingly encompassing genes currently under active investigation such as *CDKN2A, CDKN2B*, and *MTAP* - were associated with worse survival compared with deletion-negative tumors. Interestingly, this negative association was not observed in patients receiving chemo-immunotherapy.[41] Alessi et al further confirmed the role of chromosome 9p loss as an independent predictor of immunosuppressive TME and immunotherapy resistance.[39]

Biomarkers of Immunosuppressive Metabolic Pathways

Beyond genomic profiling, biomarkers of metabolic pathway dysregulation can provide insight into mechanisms sustaining an immunosuppressive TME. Among these, the tryptophan–kynurenine pathway, driven by indoleamine 2,3-dioxygenase 1 and 2 (IDO1/IDO2), represents a key tumor escape mechanism. IDO, expressed by cancer, immune, and stromal cells, catalyzes the degradation of tryptophan into immunosuppressive metabolites such as kynurenine.[42,43] This activity—detectable by IHC or quantitative real-time PCR (qPCR)—depletes tryptophan and accumulates kynurenine, thereby promoting T-cell anergy and reducing active immune cell infiltration into the TME. In NSCLC, low tryptophan, high kynurenine and 3-hydroxykynurenine levels, and an elevated kynurenine/tryptophan (Kyn/Trp) ratio, which serves as a functional readout of IDO enzymatic activity and systemic immunosuppression, have been correlated with poor prognosis and primary resistance to ICIs.[44–46]Although IDO's role as a prognostic and predictive biomarker is established, its therapeutic targeting remains controversial. Despite encouraging preclinical evidence, clinical trials with IDO1 inhibitors (eg, epacadostat) have failed to improve outcomes,[47] though novel agents and combination strategies are under investigation.

Another metabolic pathway under active investigation for its role in shaping an immunosuppressive TME is the adenosine (ADO) axis. ADO is a neurotransmitter and extracellular metabolite with potent immunosuppressive properties. Within the TME, ADO inhibits dendritic cell, NK cell, and T-cell activity, while promoting Tregs recruitment. Its production is enhanced by cellular stress and tissue damage, which induce ATP release into the extracellular space, where it is sequentially converted to ADO via either the CD39–CD73 enzymatic pathway or the CD38–NAD^+ catabolic pathway.[48,49] At low levels, ADO may exert anti-tumor effects by limiting cell proliferation. However, high extracellular ADO levels, documented in several solid tumors including NSCLC, drive immune evasion, metastasis, and angiogenesis through the CD39–CD73–A2A receptor (A2AR) axis.[50] Multiple biomarkers along this pathway are being evaluated. IHC studies report elevated CD73 expression in NSCLC as a marker of poor prognosis,[51,52] while high A2AR expression has been associated with better outcomes.[51] However, CD73 often correlates with PD-L1, and their co-expression may predict improved ICI responsiveness. In summary, CD73 may have a dual role: it reflects tumor aggressiveness as a negative prognostic factor, yet it may also serve as a positive predictive biomarker of immune response.[53] Several agents targeting this pathway—via CD39, CD73, or ADO receptor blockade—are under clinical evaluation, both alone and in combination with ICIs.[52,53]

TME Immune Infiltration as a Predictive Biomarker

An additional research avenue on emerging biomarkers focuses on techniques that allow direct assessment of immune cell infiltration into the TME. The predominant immune cell population in the TME are TILs, which belong to both the adaptive and innate arms of the immune system.[54] Previous studies have showed TILs positive impact on the clinical course of multiple cancer types, including NSCLC.[55,56] Subsequent analyses have reinforced the prognostic value of this biomarker and highlighted its predictive role in patients treated with ICI-based regimens.[54,57]

Despite their promising characteristics, the use of TILs in clinical practice remains controversial, primarily due to several limitations that can affect accurate assessment using standard methods, which rely on semiquantitative evaluation of H&E-stained slides and suffer from low reproducibility.[58] Consequently, there is growing interest in AI-based computational pathology. Notably, Rakaee et al demonstrated that AI-based assessment of TILs in NSCLC is a reliable tool; in patients with PD-L1–negative NSCLC, digitally assessed TILs were even superior to TMB in predicting response to ICIs.[57]

However, all available data on TME assessment suggest that single markers are insufficient to capture such a complex entity. Consequently, multiplexed imaging strategies are gaining increasing interest, although their application remains largely confined to the research setting, mainly due to the equipment high costs.[59] Multiplex immunohistochemistry (mIHC), which enables the simultaneous detection of multiple markers on a single formalin-fixed paraffin-embedded (FFPE) tissue section while preserving spatial context, has already shown a predictive value in patients treated with ICIs;[60,61] however, the impossibility to test more than 3–5 markers per slide is a significant limit. Multiplex immunofluorescence (mIF) builds on this approach by leveraging fluorescent dyes, which offer greater multiplexing capacity and

quantitative signal detection. Especially in combination with transcriptomic data, mIF has shown the potential to predict the response to ICI across several cancer types, including NSCLC.[22,62,63]

Other Immune Checkpoints

There is growing evidence that PD-1 and CTLA4 are not the only immune checkpoints influencing the anti-tumor immune response, although they remain the only ones targeted by clinically approved agents in NSCLC. Other receptors expressed on immune cells can contribute to T-cell exhaustion and predict resistance to ICIs. In this context, LAG-3, TIM-3, and TIGIT have attracted particular interest, both as biomarkers and as potential therapeutic targets.[64] Chronic IFN-y signaling – which is often observed in NSCLC patients – may upregulate the expression of these inhibitory receptors on T cells, thereby fostering an immunosuppressive TME. Preclinical models have demonstrated that blockade of these inhibitor receptors can restore T-cell proliferation and enhance their antitumor activity. Following the positive results of the RELATIVITY-047 trial, the anti-LAG3 monoclonal antibody relatlimab has been approved, in combination with nivolumab, for metastatic melanoma.[65] However, results with these antibodies in advanced NSCLChave been inconsistent.[66–70] Specifically, the RELATIVITY-104 trial, which tested the addition of relatlimab to the first-line standard-of -care in NSCLC, did not demonstrate a statistically significant improvement PFS, while OS data remain immature. Similarly, the addition of the anti-TIGIT antibody tiragolumab to first-line atezolizumab showed encouraging activity in the Phase II CITYSCAPE trial, conducted in untreated patients with PD-L1–positive NSCLC. In contrast, the Phase III SKYSCRAPER-01 trial, which assessed the same combination in patients with PD-L1 ≥50%, failed to meet its endpoints for both PFS and OS. Consequently, none of these agents has yet received regulatory approval for the treatment of of metastatic NSCLC. This underscores the need to validate biomarkers capable of identifying patients most likely to benefit from immune checkpoint blockade, as well as to define which ligand–receptor interactions are most critical for their immunosuppressive effects, particularly given the ongoing development of bispecific antibodies (eg, anti-PD-1/TIGIT, PD-1/LAG-3, PD-1/TIM-3) that will require careful clinical integration. These agents are designed to achieve synchronous, localized, and coordinated inhibition of both receptors on the same T cell, thereby enhancing anti-tumor activity compared with conventional co-administration of separate antibodies. Importantly, they are engineered with reduced Fc functionality, in order to minimize Fc-mediated antibody-dependent cellular cytotoxicity and antibody-dependent cellular phagocytosis that could otherwise lead to unselective depletion of TIGIT-expressing immune cells.[71–74]

Emerging Soluble Biomarkers

Due to the limitations of tissue-based biomarkers—such as the need for invasive biopsies and the challenge of tumor heterogeneity—there is increasing interest in blood-derived biomarkers. An overview of tissue- and blood-based biomarkers discussed in this review is provided in Table 2. Blood-based biomarkers encompass both circulating tumor-derived components (eg, ctDNA) and soluble biomarkers.

Table 2 Overview of Predictive and Prognostic Biomarkers for ICI Response in NSCLC

Biomarker	Biomarker Category	Definition	Predictive/Prognostic Relevance	Specimen
PD-L1 expression (TPS)	Tissue-based	Percentage of tumor cells with membranous PD-L1 staining assessed by IHC.	Predictive of benefit from ICI-based therapies; continuous variable.	Tumor tissue
Soluble / exosomal PD-L1	Blood-based (soluble)	Circulating PD-L1 detected in soluble form or on extracellular vesicles.	Exploratory; dynamic changes may correlate with outcomes.	Serum/plasma
Tumor Mutation Burden (TMB)	Tissue-based	Number of non-synonymous somatic mutations per Mb assessed by NGS.	Predictive of benefit from ICI-based therapies; continuous variable; not standardized cut-offs (utility of standardization and z-score)	Tumor tissue

(*Continued*)

https://doi.org/10.2147/ITT.S567238

Table 2 (Continued).

Biomarker	Biomarker Category	Definition	Predictive/Prognostic Relevance	Specimen
Blood TMB (bTMB)	Blood-based	Mutation burden assessed on ctDNA.	Exploratory predictive value. No standardized cut-off.	Plasma
ctDNA dynamic changes	Blood-based	Tumor-derived DNA fraction and clearance during treatment.	Early on-treatment predictor of response (in case of clearance) or resistance.	Plasma
KRAS/STK11 co-mutations or KRAS/KEAP1 co-mutations	Tissue-based	Concurrent oncogenic and tumor suppressor alterations shaping immune landscape.	Strong negative predictive value.	Tumor tissue
SMARCA4 mutations	Tissue-based	SWI/SNF complex loss affecting chromatin remodeling.	Negative predictive biomarker.	Tumor tissue
PTEN mutations	Tissue-based	Loss-of-function mutations dysregulating PI3K/AKT signaling and promoting Tregs infiltration	Negative predictive biomarker.	Tumor tissue
DNMT3A mutations	Tissue-based	Somatic loss-of-function mutations in DNMT3A, associated with distinct immunophenotype	Positive predictive biomarker.	Tumor tissue
Copy number alterations / Aneuploidy	Tissue-based	Large-scale chromosomal gains and losses.	Negative prognostic and predictive biomarker.	Tumor tissue
Chromosome 9p21 deletion	Tissue-based	Homozygous or heterozygous deletion of chromosome 9p21 (including CDKN2A, CDKN2B, MTAP, type I interferon genes)	Negative predictive biomarker independent of PD-L1 and TMB.	Tumor tissue
IDO pathway activity	Tissue-based (qPCR, IHC) / Blood-based (Kyn/Trp ratio)	Tryptophan–kynurenine metabolism mediated by IDO.	Negative prognostic and predictive biomarker.	Tumor tissue; serum
CD73 IHC expression	Tissue-based	Marker of ADO pathway	Negative prognostic biomarker, but may serve as a positive predictive biomarker of immune response when co-expressed with PD-L1	Tumor tissue
A2AR expression	Tissue-based	A2AR receptor expression on immune cells in the tumor microenvironment	Associated with better outcomes in some studies; high A2AR expression may be linked to improved immunotherapy response	Tumor tissue
Tumor-infiltrating lymphocytes (TILs) and other immune cells infiltrating TME	Tissue-based	Density and spatial distribution of immune cells in the TME.	Positive predictive biomarker	Tumor tissue
Immune checkpoints (LAG-3, TIM-3, TIGIT)	Tissue-based	Expression of inhibitory receptors associated with T-cell exhaustion.	Exploratory predictive and therapeutic biomarkers.	Tumor tissue
HLA class I diversity (HED)	Tissue-based / Blood-based	HLA class I allelic diversity and evolutionary divergence, assessed at germline or tumor level.	Higher HED associated with improved ICI outcomes.	Tumor tissue; peripheral blood
Neutrophil-to-lymphocyte ratio (NLR)	Blood-based	Ratio of absolute neutrophil count (ANC) to absolute lymphocyte count	Favorable if ≤ 5	Peripheral blood (full blood count)
Derived NLR (dNLR)	Blood-based	ANC / (WBC - ANC)	Favorable if ≤ 3	Peripheral blood (full blood count)
Platelet-to-lymphocyte ratio (PLR)	Blood-based	Ratio of absolute platelet count to absolute lymphocyte count	Not standardized	Peripheral blood (full blood count)
Lung Immune Prognostic Index (LIPI)	Blood-based	Based on dNLR and lactate dehydrogenase (LDH) levels	Good: dNLR ≤ 3 and LDH ≤ ULN; Intermediate: dNLR > 3 or LDH > ULN; Poor: dNLR > 3 and LDH > ULN	Peripheral blood (full blood count and serum biochemistry)
Circulating cytokines	Blood-based (soluble)	Baseline levels and longitudinal changes of immune mediators.	Exploratory predictors of long-term benefit.	Serum/plasma

Abbreviations: PD-L1, programmed death-ligand 1; TPS, tumor proportion score; IHC, immunohistochemistry; NGS, next-generation sequencing; ICI, immune checkpoint inhibitor; TMB, tumor mutational burden; mut/Mb, mutations per megabase; bTMB, blood tumor mutational burden; ctDNA, circulating tumor DNA; KRAS, Kirsten rat sarcoma viral oncogene homolog; SWI/SNF, SWItch/Sucrose Nonfermentable; PI3K/AKT, phosphoinositide 3-kinase/protein kinase B signaling pathway; Tregs, regulatory T cells; CDKN2A, cyclin-dependent kinase inhibitor 2A; CDKN2B, cyclin-dependent kinase inhibitor 2B; MTAP, methylthioadenosine phosphorylase; IFN, interferon; IDO, indoleamine 2,3-dioxygenase; qPCR, quantitative real-time polymerase chain reaction; Kyn/Trp, kynurenine/tryptophan ratio; ADO, adenosine; A2AR, adenosine 2A receptor; TILs, tumor-infiltrating lymphocytes; TME, tumor microenvironment; HLA, human leukocyte antigen; HED, HLA class I evolutionary divergence; NLR, neutrophil-to-lymphocyte ratio; ANC, absolute neutrophil count; WBC, white blood cell count; dNLR, derived neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; LIPI, lung immune prognostic index; LDH, lactate dehydrogenase; ULN, upper limit of normal; Kyn, kynurenine; Trp, tryptophan.

 https://doi.org/10.2147/ITT.S567238

Systemic Inflammation Scores as Prognostic Markers

Within this framework, markers of systemic inflammatory response have drawn particular attention, given the key role of inflammation in modulating the efficacy of systemic therapies, including ICIs. In NSCLC, several affordable and reproducible pretreatment blood-based biomarkers—such as the neutrophil-to-lymphocyte ratio (NLR), lactate dehydrogenase (LDH), platelet-lymphocyte ratio (PLR) and the lung immune prognostic index (LIPI) score—are routinely used to predict clinical outcomes, due to their accessibility.[75–79]

The prognostic relevance of these biomarkers is well established across multiple treatment modalities, including ICI-based regimens.[76,80,81] The LIPI prognostic score in NSCLC has been further validated by a meta-analysis of more than 8,000 patients from 8 clinical studies, which consistently showed poorer survival outcomes in patients with higher LIPI scores, regardless of treatment modality.[82] In fact, the LIPI score integrates dNLR and LDH, reflecting systemic inflammation, immune activation and tumor burden. An elevated dNLR is associated with neutrophil-driven immunosuppression and relative lymphopenia, whereas LDH reflects tumor metabolism and hypoxia-related aggressiveness. Together, these parameters provide a biologically plausible explanation for the prognostic value of LIPI in immunotherapy-treated patients. Notably, the prognostic value of LIPI is not restricted to NSCLC: intermediate or poor pretreatment scores were also associated with worse outcomes in patients with extensive-stage SCLC treated with chemoimmunotherapy.[83] Together, these findings highlight LIPI as a robust prognostic marker across both treatment modalities and lung cancer histologies.

A recent Chinese trial has sought to specifically explore the association between these biomarkers and immunotherapy outcomes.[84] Among 202 patients treated with various immunotherapy agents, a «good» LIPI score was significantly associated with prolonged progression-free survival (PFS >24 months). Similarly, lower NLR values were observed in long-responder patients, reflecting a less inflammatory systemic profile. These findings support the hypothesis that reduced systemic inflammation may help preserve circulating lymphocytes and promote TILs, thereby enhancing anti-tumor immune responses. The authors suggested that integrating blood-based biomarkers with clinical characteristics (eg, number and location of metastases) and cytokine profiles could improve patient stratification and help identify those most likely to benefit from long-term immunotherapy.

Interestingly, not only baseline levels but also the evolution of these biomarkers during treatment has shown clinical relevance. An Italian retrospective study demonstrated that improvements in the LIPI score during treatment (eg, from intermediate to good) were associated with a higher likelihood of developing immune-related adverse events (irAEs), potentially reflecting enhanced immune activation[79] and a more reactive immune system, with overlapping mechanisms between antitumor immunity and autoimmunity. Similarly, post-treatment deterioration of inflammatory markers—such as increases in NLR or worsening of the LIPI score—has been linked to poorer PFS and OS.[85]

Liquid Biopsy and ctDNA

Liquid biopsy (LB) is a non-invasive diagnostic tool used to isolate tumor-derived components from biological fluids, such as blood, urine, cerebrospinal fluid, and pleural effusions.[86] It is easily reproducible, allowing for longitudinal monitoring and supporting an increasingly personalized approach. The concept of LB encompasses the analysis of circulating tumor cells (CTCs) as well as tumor-derived molecules, including circulating tumor DNA (ctDNA), circulating tumor RNA (ctRNA), microRNAs (miRNAs), and exosomes.

Among these, ctDNA stands out as a promising biomarker for ICIs in NSCLC, because it is highly specific and carries tumor genetic alterations, capturing tumor heterogeneity more effectively than a single tissue biopsy. Detection methods can be either targeted, via panels tailored to known mutations, or untargeted, via tumor-agnostic approaches employing high-throughput NGS to assess broader genomic alterations, including WES or whole-genome sequencing (WGS).[87] A key limitation of ctDNA-based monitoring is that not all tumors are “shedders,” meaning that ctDNA may be undetectable in some patients. In this context, assessing the ctDNA tumor fraction—the percentage of ctDNA within the total cell-free DNA (cfDNA)—may help distinguish between truly negative results and non-informative ones. However, tumor fraction is not yet routinely applied in clinical practice, as defining optimal cutoffs, ensuring assay sensitivity, and addressing associated costs remain significant challenges.[88] Nevertheless, ctDNA analysis has become an

important clinical tool in NSCLC. Since 2016, the FDA has approved ctDNA testing for *EGFR* mutation detection, thereby establishing liquid biopsy as a standard method for molecular profiling when tissue is unavailable or insufficient. Multiple studies have demonstrated its high mutation-detection rate and its ability to reduce the time to treatment initiation.[89,90] Moreover, ctDNA allows real-time monitoring of disease evolution. Decrease or clearance of ctDNA during treatment with ICIs (with or without chemotherapy) has been associated with improved outcomes.[91,92] Therefore, ctDNA monitoring may hold a key application in metastatic NSCLCs when selecting which patients should escalate/de-escalate treatment during ICI or continue ICI longer than 2 years.[93,94] Notably, a retrospective study by Besse et al suggested that ctDNA tumor fraction may predict early disease progression and help identify patients more likely to benefit from chemotherapy in addition to immunotherapy.[95] Finally, ctDNA may serve in the early disease setting to predict who will experience pathological complete response after chemotherapy + ICI as neoadjuvant therapy or who will have improved prognosis on ICI monotherapy in the adjuvant setting, with the ultimate goal to adjust treatment based on whether ctDNA is detected or not after curative-intent therapy, either surgery or definitive chemoradiotherapy.[96,97] Overall, ctDNA dynamics reflect tumor burden and disease biology, and early decreases or clearance during treatment are increasingly recognized as markers of effective immune-mediated tumor control. This supports a potential prognostic—and possibly predictive—role for ctDNA beyond static baseline measurements.

As a derivative of ctDNA, blood TMB (bTMB) can also be assessed; prior analyses suggest that bTMB may serve as an independent predictive marker of ICI efficacy. However, the role of its longitudinal assessment remains more controversial,[98] because bTMB reflects only the fraction of tumor DNA released into the circulation and may be influenced by tumor burden and shedding dynamics.

Other Soluble Biomarkers

Circulating cytokines play diverse roles in immunomodulation, and their concentrations may help clinicians distinguish between good and poor immunotherapy responders. For example, peripheral blood samples from 19 metastatic NSCLC patients were collected before treatment and at 6 weeks after the start of immunotherapy. The study found that increasing CCL11 levels (which inhibit angiogenesis and induce eosinophil migration and activation) and decreasing IL-1Ra (an anti-inflammatory cytokine) and IL-17A (a cytokine which favors cancer cell invasion and resistance to anti-PD-1 therapy) levels were associated with long-term responders (PFS > 24 months). The kinetics of circulating cytokines, when combined with clinical features and other biomarkers, may help identify patients likely to benefit from prolonged treatment responses.[84]

Researchers are also increasingly interested in soluble PD-L1 (sPD-L1) and exosomal PD-L1 (exoPD-L1), as these can reduce antitumor efficacy. The prognostic role of these biomarkers remains debated: elevated levels generally correlate with worse PFS or OS, although, for example, increases in exoPD-L1 during treatment may indicate favorable outcomes.[99,100] Collectively, these biomarkers offer practical advantages for risk stratification, early treatment monitoring, and the potential prediction of toxicity. However, despite growing interest, their routine implementation in clinical practice remains limited, as mechanistic evidence is still incomplete and current findings largely rely on retrospective data that require prospective validation.

HLA-I Heterozygosity and Evolutionary Divergence

$CD8^+$ T cell–mediated cytotoxicity against cancer cells depends on efficient tumor antigen presentation by human leukocyte antigen (HLA) molecules. Based on this rationale, HLA class I heterozygosity and diversity have attracted interest as potential predictive biomarkers across multiple cancer types.[101,102] Heterozygosity at HLA loci may result in greater allelic polymorphism, enabling the presentation of a broader repertoire of neoantigens compared with homozygosity. However, heterozygosity alone does not necessarily confer functional diversity, which may represent the actual driver of enhanced responsiveness to immune checkpoint inhibition. To address this limitation, Chowell et al proposed the concept of germline HLA-I evolutionary divergence (HED),[103] a quantitative metric that captures the functional dissimilarity between the two alleles at each HLA-A, HLA-B, and HLA-C locus. HED is calculated using the Grantham distance[104] between the peptide-binding domains of paired alleles, with higher HED values reflecting greater divergence in peptide-binding properties. From a biological standpoint, higher HED may enable the presentation of a more diverse

set of tumor-derived antigens, thus broadening the repertoire of T-cells capable of recognizing and killing malignant cells. In their analysis, Chowell et al demonstrated that in patients with melanoma or NSCLC treated with ICIs, higher HED correlated with better outcomes and served as an independent biomarker in Cox multivariable analysis.[103]

Potentially, HLA diversity and HED can be evaluated through blood-based germline HLA genotyping, thus avoiding the need for biopsies or tumor tissue. However, evidence suggests that tumor cells may acquire (epi)genetic alterations leading to HLA-I downregulation, impaired or reduced antigen presentation, and loss of heterozygosity.[105,106] Differences in study design, including the type of biological specimen analyzed and whether only HLA heterozygosity or also HED was assessed, may partly explain why some investigations have reported less consistent results than those of Chowell et al regarding the robustness of HLA as a predictive biomarker.[102,107] Overall, while HLA diversity and HED are promising and remain under active investigation, they are not easily integrated into routine clinical practice due to multiple challenges, including cost, test availability, and the choice of the appropriate specimen.

Host Factors and Response to Immunotherapy

There is growing recognition that host-related characteristics substantially influence the efficacy of ICIs. Baseline features such as history of tobacco exposure, sex at birth, and body mass index (BMI) have emerged as clinically relevant variables, with potential implications for treatment outcomes. Taking into account host determinants may therefore support a more individualized approach to immunotherapy in NSCLC.

Tobacco Exposure and Immunotherapy Outcomes

History of tobacco exposure is a key determinant of immunotherapy efficacy in NSCLC. Patients without a history of tobacco use account for 10–25% of cases worldwide, with a higher prevalence among women in Asian and Middle Eastern populations.[108,109] These tumors generally have a more favorable prognosis, largely due to the frequent presence of selected AGAs (eg EGFR, ALK, ROS1, RET, NTRK, ERBB2), for which effective targeted therapies are available. In contrast, they typically exhibit molecular and microenvironmental features associated with reduced benefit from ICIs, including lower TMB, a higher transitions/transversions (Ti/Tv) ratio, and a less immunogenic, more suppressive TME.[108]

Clinical evidence supports this biological observation. Several registrational trials of ICIs have reported lower benefit in this population,[110,111] and multiple retrospective analyses have consistently shown a positive association between smoking history and ICI efficacy, even in tumors with PD-L1 expression ≥50%. For example, Li et al conducted a prospective analysis at Princess Margaret Cancer Center showing higher response rates in patients with tobacco exposure, irrespective of PD-L1 status.[112] Similarly, Gainor et al reported a favorable trend in both progression-free survival and duration of response among patients with PD-L1 ≥50%.[113] Cortellini et al also observed that, within a PD-L1 ≥50% cohort treated with pembrolizumab, tobacco exposure was associated with a lower risk of progression and death. Importantly, this effect was not explained by a more aggressive biology of tumors in patients without tobacco exposure, as the opposite trend was observed in contemporaneous cohorts treated with chemotherapy.[114]

Given the suboptimal outcomes with PD-(L)1 blockade in NSCLC patients without a history of tobacco exposure, treatment intensification strategies are being explored. Subgroup analyses from multiple prospective trials, as well as a recent meta-analysis by Luo et al, indicate a benefit from chemo-immunotherapy combinations,[115–117] while the benefit of the addition of anti-CTLA-4 agents remains controversial.[116,118,119] Further research is needed to refine predictive biomarkers and optimize treatment approaches for this understudied subgroup.

The Interplay Between Body Composition and Sex in Shaping Immunotherapy Response

Body composition (BC) and sex modulate the immune system and influence the antitumor response,[120,121] potentially affecting immunotherapy efficacy. These parameters reflect patients' metabolic and hormonal background and can shape both immune function and the TME. Understanding how BC modification and sex-specific factors interact with the tumor

and the immune system may provide valuable insight into the variability of ICI outcomes, ultimately helping to optimize patient selection for immunotherapy.

Obesity has long been recognized as a risk factor for solid tumors and is associated with poorer outcomes among cancer patients.[122] In fact, obesity exerts multifaceted effects on tumorigenesis across different cancer types: adipose tissue (AT) acts as an endocrine organ through estrogen production,[123,124] activation of insulin–IGF-1 signaling,[125] and secretion of adipokines that drive chronic inflammation.[126,127] Whitin the TME, obesity impairs immunity by promoting macrophage polarization toward the M2 phenotype, expanding myeloid-derived suppressor cells (MDSCs), and inducing CD8+ T cell exhaustion.[128–131] Leptin-driven PD-1 overexpression on CD8+ T cells, mediated through STAT3 signaling, further contributes to this dysfunctional state.[132]

Interestingly, this immunosuppressive environment may enhance tumor susceptibility to checkpoint inhibition: preclinical evidence has shown that in obese mouse models, PD-1 blockade restored T cell effector function and enhanced tumor regression compared to lean controls.[132,133] This paradox, known as the "obesity paradox," suggests that obesity both impairs immunosurveillance and simultaneously primes tumors for ICI efficacy.

Despite its association with chronic inflammation, recent evidence indicates that patients with a BMI ≥ 25 kg/m2 may experience improved survival with ICIs compared with those with normal BMI.[134]

In NSCLC, several clinical studies have confirmed that overweight and obese patients achieve better outcomes with ICIs. Pooled trial analyses demonstrated longer OS in patients with BMI ≥25 kg/m^2 treated with atezolizumab, with the strongest effect in those with BMI ≥30 kg/m^2.[135] Similarly, in retrospective cohorts of patients with PD-L1 ≥50%, baseline obesity was associated with higher response rates and longer PFS and OS in patients receiving pembrolizumab, but not in those treated with chemotherapy.[136] Meta-analyses further supported this survival advantage in obese patients,[137] whereas large real-world datasets challenged these findings, showing no independent prognostic role for BMI overall.[138–140]

Altogether, these findings remain inconclusive, underscoring the need for future studies to incorporate more accurate metrics of BC when evaluating immunotherapy outcomes.

Indeed, the impact of obesity on immunotherapy efficacy cannot be fully understood without considering sex-related dimorphisms. Men and women differ in fat distribution: men predominantly accumulate visceral adipose tissue, while women store more subcutaneous fat before menopause, shifting toward visceral fat in later life. These differences in BC are immunologically relevant, as adiposity distribution influences systemic inflammation and long-term immune memory.[141]

Several studies suggest that the "obesity paradox" in immunotherapy may be more pronounced in men. In patients with advanced melanoma treated with ICIs, obesity was associated with better survival in male but not in females.[142,143] Preclinical data reinforce this sex-specific effect: in murine tumor models of melanoma, obese males responded significantly better to PD-1 blockade, whereas obese females did not experience similar benefit. Interestingly, ovariectomy in female mice led to both weight gain and restored sensitivity to ICIs,[144] pointing to sex hormones as critical mediators of the relationship between AT and immunotherapy response.

Beyond adiposity, intrinsic sex-related immune differences also influence ICI outcomes. Meta-analyses have suggested greater ICI benefit in men when ICIs are given as monotherapy, whereas women appear to derive more benefit from chemoimmunotherapy combinations.[145,146] Moreover, machine-learning models integrating clinical, genomics and immune features have similarly identified male sex as a positive predictor of ICI response.[147]

Transcriptomic and molecular analyses of NSCLC tumors have revealed stronger immune infiltration in women, accompanied by higher expression of inhibitory checkpoints and immunosuppressive populations, consistent with adaptive resistance. In contrast, men displayed a more immune-excluded phenotype with frequent HLA loss of heterozygosity.[121]

In this contest, hormonal signalling plays a central role in modulating response to ICIs. Androgen receptor (AR) activation promotes T cell exhaustion,[148] while evidence while evidence from prostate cancer suggests that AR inhibition synergizes with PD-1 blockade to restore T cell cytotoxicity activity.[149,150] Estrogen receptor signalling drives M2 macrophage polarization and suppresses CD8+ effector activity, whereas pharmacological blockade with fulvestrant has been shown to enhance ICI efficacy in preclinical models.[151]

Aging further complicates this interplay: hormonal decline, sarcopenia, and chronic inflammation impair immune surveillance and predict poor immunotherapy outcomes.[148,152,153]

Taken together, these findings indicate that BMI and sex are not independent, but rather interconnected, host-related factors shaping immunotherapy outcomes. Obesity promotes systemic inflammation and immune dysfunction but, paradoxically, may enhance responses to PD-1/PD-L1 blockade. The magnitude and direction of this effect appear to differ by sex, likely reflecting hormonal regulation and differences in adipose distribution. The distinct metabolic and hormonal environments of men and women influence the quality and extent of ICI benefit. Integrating BC measures, sex-specific immune features, and hormonal status into biomarker development is essential to achieve more precise stratification of patients undergoing immunotherapy.

AI-Based Integrative Models for Predicting Immunotherapy Response

AI in oncology refers to the use of computational algorithms to analyze large and complex datasets, with the goal of identifying patterns, classifying outcomes, or generating predictions that can inform clinical decision-making. Within AI, machine learning (ML) refers to algorithms that improve performance by learning from data, while deep learning (DL) represents a subset of ML that uses multilayer neural networks to automatically extract features and recognize complex patterns. In the last years, AI has been successfully employed in diagnostics, therapeutics, and prognostic predictions in several neoplasms.[87,154,155]

A major challenge in the identification of robust immunotherapy biomarkers is the multifactorial nature of tumor immunogenicity, which is shaped by both tumor-intrinsic and tumor-extrinsic determinants. AI offers the advantage of integrating these diverse variables, while ML approaches further support the development of predictive scoring systems. By employing TCGA data and ML, Charoentong et al described the immunophenoscore, a pan-cancer immune gene signature designed to identify patients more likely to benefit from immunotherapy, thus underscoring the possible contribution of AI in this setting.[156] More recently, Christopoulos et al applied a machine learning–based algorithm to classify patients according to their proteomic profile. The test, performed prior to initiation of ICI-based therapy (either alone or in combination with chemotherapy) in metastatic NSCLC, stratified patients into PROphet-negative and PROphet-positive groups. Notably, the PROphet test outperformed PD-L1 in identifying patients more likely to benefit from treatment escalation with the addition of chemotherapy.[157] In line with these efforts, SCORPIO (Standard Clinical and labOratory featuRes for Prognostication of Immuno therapy Outcomes), a ML system that utilizes routine blood tests and clinical characteristics, was developed and trained on data from 1628 patients who received ICI at Memorial Sloan Kettering Cancer Center for 17 different cancer types. Internal and external tests validation tests showed that SCORPIO can outperform TMB in predicting ICI outcomes, potentially paving the way for the integration of AI-based predictors of response into clinical practice.[158]

Conclusion

In recent years, significant efforts have been made to develop novel therapeutic agents, in order to overcome both acquired and primary resistance to ICI: ADCs, bispecific antibodies, vaccines, and adoptive cell therapies have been tested in several trials and are still under active investigation. However, many trials aimed at enhancing ICI efficacy—often through combination strategies with the aforementioned therapeutic approaches—have shown controversial results. A common limitation of these studies has been the lack of appropriate patient selection. It is therefore reasonable to hypothesize that this challenge in approving new therapeutic strategies likely reflects the scarcity of robust predictive biomarkers, which would allow clinicians to identify patients who need treatment escalation and to select the most appropriate strategy. As extensively discussed, PD-L1 and TMB, despite being approved by multiple regulatory authorities and widely used in clinical practice, present important biological and technical limitations. This has fueled increasing interest in the identification of novel and emerging predictors of response to immunotherapy. Overall, the biomarkers discussed in this review can be broadly categorized into tissue-based, soluble and liquid biopsy–derived, host-related, and AI-driven integrative approaches, each capturing distinct and complementary aspects of tumor–immune interactions. While several of these biomarkers have shown predictive potential, none has proven sufficient as a standalone tool, highlighting the need for multidimensional strategies to refine patient stratification.

Given the complexity of both the tumor and the immune system, it is probably necessary to integrate multiple variables that describe tumor genomic profile, TME characteristics, and baseline immune status. For this reason, AI—capable of analyzing multiple variables simultaneously—should be considered a helpful tool, as long as it is guided by the expertise of clinicians. In this context, AI-based models may facilitate the integration of heterogeneous biomarker signals and support the translation of complex biological information into clinically actionable frameworks. Future efforts should focus on integrating predictive biomarkers and using them to design clinical trials capable of effectively pave the way for tailored ICI-based treatments.

Acknowledgments

Dr. Eleonora Gariazzo is supported by Fondazione Avanti Tutta.

Dr. Valentina Santo was supported by an American-Italian Cancer Foundation Post-Doctoral Research Fellowship, year 2025–2026.

Disclosure

Alessio Cortellini reports personal fees from Astrazeneca, personal fees from MSD, personal fees from Roche, personal fees from BMS, personal fees from Yuhan corporation, outside the submitted work. Biagio Ricciuti reports being on the advisory board for AstraZeneca, Regeneron, BMS, AbbVie, Lilly, J & J, and Caris Life, during the conduct of the study. Giulio Metro reports speaker's fee from Amgen, congress travel fee from Takeda, speaker's fee from AstraZeneca, during the conduct of the study. The authors report no other conflicts of interest in this work.

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