Predictive Biomarkers for Immunotherapy in Lung Cancer: Perspective From the International Association for the Study of Lung Cancer Pathology Committee

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ABSTRACT

Immunotherapy including immune checkpoint inhibitors (ICIs) has become the backbone of treatment for most lung cancers with advanced or metastatic disease. In addition, they have increasingly been used for early stage tumors in neoadjuvant and adjuvant settings. Unfortunately, however, only a subset of patients experiences meaningful response to ICIs. Although programmed death-ligand 1 (PD-L1) protein expression by immunohistochemistry (IHC) has played a role as the principal predictive biomarker for immunotherapy, its performance may not be optimal, and it suffers multiple practical issues with different companion diagnostic assays approved. Similarly, tumor mutational burden (TMB) has multiple technical issues as a predictive biomarker for ICIs. Now, ongoing research on tumor- and host immune-specific factors has identified immunotherapy biomarkers that may provide better response and prognosis prediction, in particular in a multimodal approach. This review by the International Association for the Study of Lung Cancer Pathology Committee provides an overview of various immunotherapy biomarkers, including updated data on PD-L1 IHC and TMB, and assessments of neoantigens, genetic and epigenetic signatures, immune microenvironment by IHC and transcriptomics, and microbiome and pathologic response to neoadjuvant immunotherapies. The aim of this review is to underline the efficacy of new individual or combined predictive biomarkers beyond PD-L1 IHC and TMB.

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Keywords: Predictive biomarkers; Immunotherapy; Lung cancer; PD-L1; TMB; Neoadjuvant therapy

Introduction

Lung cancer is the leading cause of cancer-related death worldwide. Its poor prognosis is historically attributed to difficulty in early detection and its low response rates to conventional chemotherapy with or without radiation therapies. Nevertheless, critical progress has been made in the past decade to substantially improve survival of metastatic non-small cell lung cancer (NSCLC). In particular, immune checkpoint inhibitors (ICIs) including the programmed cell death protein-1 (PD-1)/programmed death-ligand 1 (PD-L1) axis blockade, in either first- or second-line settings and irrespective of the histological subtypes of NSCLC, have led to unprecedented prolonged survival for a subset of patients. To date, eight anti-PD-1 or PD-L1 antibodies (pembrolizumab, nivolumab, atezolizumab, durvalumab, cerdulimab-rwlc, sintilimab, tislelizumab, and camrelizumab) have been approved globally or in some countries for treatment of NSCLC, either as monotherapy or in combination with chemotherapy and or an anti-CTLA-4 agent.

Although PD-L1 immunohistochemistry (IHC) has been used as a companion diagnostic for anti-PD-1 or PD-L1 monotherapy or nivolumab and ipilimumab combination therapy, it is known to be an imperfect predictive biomarker. Similarly, tumor mutational burden (TMB) has multiple technical issues as a predictive biomarker, although the FoundationOne CDx assay has been approved by the Food and Drug Administration (FDA) as a companion diagnostic for second-line pembrolizumab monotherapy for solid tumors including lung cancer with high TMB.

After the publication of a perspective on PD-L1 testing for lung cancer, on TMB, and on pathologic assessment of lung cancer resection specimens after neoadjuvant therapy, this article from the International Association for the Study of Lung Cancer (IASLC) Pathology Committee provides an overview of various biomarkers that may help in predicting ICI response in NSCLC, featuring updated information on PD-L1 IHC and TMB, and neoantigens, genetic and epigenetic signatures, immune microenvironment assessments by IHC, transcriptomic analyses and novel technologies, and microbiome composition. Assessments of pathologic response to neoadjuvant immunotherapies, which are considered predictive of overall survival, are also discussed. This review emphasizes the notion that a combination of predictive biomarkers outperforms the use of individual marker approach, and might help implement a multimodal approach in clinical practice.

PD-L1 as Predictive Biomarker for Immunotherapy in Lung Cancer

PD-L1 protein expression has emerged as a clinically useful biomarker for treatment decisions regarding immunotherapy (immuno-oncology [IO]). Several studies have led to approvals by regulatory agencies on the basis of PD-L1 expression (Table 1). Although initially there was a confusion about the comparability of different PD-L1 IHC assays used in clinical trials, several studies including the IASLC “PD-L1 Blueprint Project” compared the different assays and found three assays very similar in expression.
very high protein expression (TPS ≥ 1%)

In addition, retrospective analyses have revealed that high PD-L1 expression (approximately 30% of NSCLC) is a predictive marker for IO as first-line treatment versus chemotherapy. Many guidelines point to IO alone or in combination with chemotherapy as the treatment of choice in high PD-L1 expressors as revealed in the CheckMate 141 trial (NCT03793179), many guidelines point to IO as the treatment of choice in high PD-L1 expressors. However, high PD-L1 expression does not necessarily indicate a favorable outcome with PD-L1 monotherapy, as revealed in the CheckMate 227 study, no or low PD-L1 expression indicated a favorable outcome with PD-L1 monotherapy.

Whether there is a difference in the predictive value on the basis of histological subtypes, a retrospective analysis of a large number of patients indicates no predictive value of PD-L1 expression in squamous cell carcinoma in contrast to adenocarcinoma. Furthermore, in contrast to NSCLC, SCLC has in general low PD-L1 expression. So far, no predictive role of PD-L1 expression has been found related to IO in SCLC.

Although the above-mentioned predictive paradigm seems relatively simple, many issues remain. First, PD-L1 expression is spatially and temporally heterogeneous and may vary between tumor sites (primary versus metastases). Second, the continuous distribution of PD-L1 expression makes the determination of a reliable binary cutpoint difficult. Last, but not least, it needs to be formally evaluated whether combination of PD-L1 IHC with other markers such as TMB, gene expression profiling (transcriptomic signatures), and multiplex IHC and multiplex immunofluorescence (mIF) for tumor microenvironment (TME) component analyses may be more predictive than PD-L1 IHC alone. For instance, we already know that high PD-L1 expression does not significantly overlap with high TMB and that combination of PD-L1 expression and TMB might give a better prediction than one biomarker alone.
TMB, Neoantigens, Genetic and Epigenetic Signatures

In September 2020, our group published a review article on the promises and challenges of TMB as an immunotherapy biomarker. The recognized challenges included the following: (1) identification of therapies whose response was best informed by TMB status; (2) robust definition of a predictive TMB cutpoint; (3) standardization of sequencing panel size and design; and (4) the need for robust technical and informatics rigor to generate precise and accurate TMB measurements across different laboratories. Nevertheless, on June 16, 2020, the FDA granted an accelerated approval of pembrolizumab for the treatment of adult and pediatric patients with unresectable or metastatic solid tumors with high tumor tissue mutational burden (tTMB-H, as defined by ≥10 mutations/megabase [mut/Mb]) determined by an FDA-approved test, who have progressed after previous treatment and who have no satisfactory alternative treatment options. Importantly, the FDA-approved TMB test referred to the FoundationOne CDx assay (Foundation Medicine, Inc.), which was approved on the same day as the companion diagnostic for pembrolizumab in this setting. This approval was based on the results of KEYNOTE-158 (NCT02628067), a phase 2 multicohort, open-label, nonrandomized study involving 10 types of advanced incurable solid tumors including SCLC. Among 790 patients with assessable tTMB, 102 (13%) had tTMB-H, and their overall response rate was 29% (95% confidence interval [CI]: 21–39) versus 6% (95% CI: 5–8) for the non–tTMB-H group. Impressively, 57% and 50% of patients who responded had response duration of more than or equal to 12 months and 24 months, respectively. Among the 76 patients with SCLC included, 34 (45%) had tTMB-H. The overall response rate for tTMB-H and non–tTMB-H patients with SCLC were 29% and 9.5%, and median overall survivals were 9.4 (95% CI: 5.6–19.1) and 6.3 (95% CI: 3.9–7.7) months, respectively. This result is consistent with the results of the TMB study on the efficacy of nivolumab or nivolumab plus ipilimumab in previously treated patients with SCLC who were accrued to the CheckMate 032 trial, as discussed in our previous review. Nevertheless, assessment of blood TMB in IMpower133 trial revealed the lack of predictiveness of this marker (at either 10 or 16 mut/Mb) for the efficacy of atezolizumab plus chemotherapy in extensive-stage SCLC.

To date, assessment of TMB as a predictive marker for ICI therapy in NSCLC trials has largely been retrospective and exploratory, except in CheckMate-227. Overall, the data suggested that high tTMB was consistently associated with greater survival benefit from anti–PD-1 or PD-L1 monotherapy versus standard-of-care chemotherapy, whereas such benefit was not apparent in low tTMB patients (Table 2). Nevertheless, this difference was not apparent when ICI is combined with chemotherapy or when dual ICI is compared with single ICI therapy (MYSTIC trial). As previously discussed, despite initial optimistic results in CheckMate 227 that high TMB (≥10 mut/Mb) could be predictive for nivolumab plus ipilimumab combination therapy, the subsequent overall survival data suggested that TMB was prognostic rather than predictive, leading to eventual FDA approval of this ICI combination therapy as first-line treatment in patients with metastatic NSCLC without EGFR/ALK aberration on the basis of the PD-L1 (≥1%) status only.

Limited data are available on the role of plasma or blood TMB (bTMB) as predictive marker for ICI (Table 3). In the MYSTIC trial, although high bTMB (≥20 mut/Mb) is associated with improved OS for durvalumab plus tremelimumab versus chemotherapy, this effect was not found in patients with less than 20 mut/Mb bTMB, but the analysis was retrospective and exploratory. When this cutoff was tested in the BR.34 trial that compared the efficacy of durvalumab plus tremelimumab with versus without platinum-doublet chemotherapy, bTMB was found to have no differential treatment effect on OS. When ICI combined with chemotherapy was compared with chemotherapy alone, there have been conflicting results as to whether high bTMB can predict differential treatment effects on survival.

Targeted gene panels are now routinely used for clinical identification of driver mutations using next-generation sequencing technology in many countries, but these panels vary widely in the number and composition of genes covered. Several in silico and actual panel sequencing studies have revealed strong correlation of TMB estimated using these panels compared with whole exome sequencing, and panels of greater than 1.1 Mb from anti-
Table 2. Tissue-Based Evaluation of TMB as Predictive Biomarker for ICI Therapies in Phase 3 Trials Involving NSCLC

<table>
<thead>
<tr>
<th>Line of Tx</th>
<th>Clinical Trial</th>
<th>Analysis</th>
<th>Study Therapy</th>
<th>Reference Therapy</th>
<th>Assay</th>
<th>Cutpoint</th>
<th>Survival</th>
<th>Ref</th>
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<td>1L</td>
<td>CM-26</td>
<td>E</td>
<td>Nivo</td>
<td>Chemo</td>
<td>WES</td>
<td>243 mut/exome</td>
<td>PFS</td>
<td>0.62 (0.38-1.00) 1.82 (1.30-2.55) 160</td>
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<td>1L</td>
<td>KN-042</td>
<td>E</td>
<td>Pembro</td>
<td>Chemo</td>
<td>WES</td>
<td>175 mut/exome</td>
<td>PFS</td>
<td>0.75 (0.59-0.95) 1.27 (1.04-1.55) 161</td>
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<td>1L</td>
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<td>E</td>
<td>Durva</td>
<td>Chemo</td>
<td>F1CDx</td>
<td>10 mut/Mb</td>
<td>OS</td>
<td>0.70 (0.47-1.06) 1.26 (0.90-1.77) 29</td>
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<td>Pembro</td>
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<td>WES</td>
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<td>PFS</td>
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<td>Chemo</td>
<td>F1</td>
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<td>0.49 (0.19, 1.3) 1.28 (0.77, 2.12) 162</td>
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<td>+ anti-CTLA-4 vs. chemo</td>
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<td>CM-227</td>
<td>P</td>
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<td>Chemo</td>
<td>F1CDx</td>
<td>10 mut/Mb</td>
<td>PFS</td>
<td>0.58 (0.41-0.81) 1.07 (0.84-1.35) 27,28</td>
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<td>Anti-PD1</td>
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<td>E</td>
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<td>Chemo</td>
<td>OncoScreen</td>
<td>10 mut/Mb</td>
<td>PFS</td>
<td>0.44 (0.27-0.72) 0.57 (0.36-0.91) 32</td>
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<td>0.74 (0.51-1.08) 0.75 (0.55-1.02) 33</td>
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<td>E</td>
<td>Durva + Treme</td>
<td>Durva</td>
<td>F1CDx</td>
<td>10 mut/Mb</td>
<td>OS</td>
<td>1.00 (0.65-1.54) 1.09 (0.79-1.50) 29</td>
</tr>
</tbody>
</table>

*Enrolling squamous cell carcinoma only.

Tx, therapy; L, line; E, exploratory; P, prospective; ICI, immune checkpoint inhibitor; chemo, chemotherapy; nivo, nivolumab; pembro, pembrolizumab; durva, durvalumab; atezo, atezolizumab; ipi, ipilimumab; treme, tremelimumab; tisle, tislelizumab; F1, FoundationOne; F1CDx, FoundationOne CDx; PD-1, programmed cell death protein 1; PFS, progression-free survival; TMB, tumor mutational burden; OS, overall survival; PD-1, programmed cell death protein 1; WES, whole exome sequencing; KN, KeyNote; CM, CheckMate.
<table>
<thead>
<tr>
<th>Line of Tx</th>
<th>Clinical Trial</th>
<th>Study Therapy</th>
<th>Reference Therapy</th>
<th>Assay (Cutpoint)</th>
<th>Survival</th>
<th>Hazard Ratio (95% Confidence Interval)</th>
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<td>Atezo</td>
<td>Chemo</td>
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<td>OS</td>
<td>0.64 (0.44-0.92) 0.65 (0.52-0.81) 23</td>
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<td>Durva</td>
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<td>F1CDx (20 mut/Mb)</td>
<td>PFS</td>
<td>0.77 (0.52-1.13) 1.19 (0.94-1.50) 29</td>
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<td>0.72 (0.50-1.05) 0.93 (0.74-1.16) 29</td>
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<td>0.49 (0.32-0.74) 1.16 (0.93-1.45) 29</td>
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<td>1L MYSTIC</td>
<td>Durva + Treme</td>
<td>Durva</td>
<td>F1CDx (20 mut/Mb)</td>
<td>PFS</td>
<td>0.76 (0.50-1.15) 1.26 (1.02-1.57) 29</td>
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<td>OS</td>
<td>0.74 (0.48-1.11) 1.22 (0.98-1.52) 29</td>
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<td>Anti-PD-1 ± anti-CTLA-4 + chemo vs. chemo</td>
<td>1L RATIONALE-307\textsuperscript{2}</td>
<td>Tisle + Chemo</td>
<td>Chemo</td>
<td>OncoScreen (6 mut/Mb)</td>
<td>PFS</td>
<td>0.30 (0.13-0.67) 0.63 (0.25-1.61) 32</td>
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<td>OncoScreen (8 mut/Mb)</td>
<td>PFS</td>
<td>0.33 (0.14-0.75) 0.55 (0.22-1.34) 32</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>OncoScreen (10 mut/Mb)</td>
<td>PFS</td>
<td>0.30 (0.11-0.82) 0.51 (0.23-1.14) 32</td>
</tr>
<tr>
<td></td>
<td>1L CKM-9LA</td>
<td>Nivo + Ipilimumab</td>
<td>Chemo</td>
<td>Guardant OMNI (16 mut/Mb)</td>
<td>PFS</td>
<td>0.60 (0.42-0.86) 0.73 (0.55-0.96) 33</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>OS</td>
<td>0.55 (0.39-0.78) 0.78 (0.60-1.00) 33</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Guardant OMNI (20 mut/Mb)</td>
<td>PFS</td>
<td>0.54 (0.35-0.84) 0.74 (0.57-0.95) 33</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>OS</td>
<td>0.48 (0.32-0.73) 0.78 (0.62-0.99) 33</td>
<td></td>
</tr>
<tr>
<td>Anti-PD-L1 ± anti-CTLA-4 + chemo + ICI</td>
<td>1L BR.34</td>
<td>Durva + Treme + chemo</td>
<td>Durva + Treme</td>
<td>Guardant OMNI (20 mut/Mb)</td>
<td>PFS</td>
<td>0.96 (0.54-1.71) 0.58 (0.42-0.81) 31</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>OS</td>
<td>0.98 (0.53-1.80) 0.81 (0.59-1.12) 31</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a}Enrolling squamous cell carcinoma only.

Tx, therapy; L, line; chemo, chemotherapy; ICI, immune checkpoint inhibitor; durva, durvalumab; atezo, atezolizumab; treme, tremelimumab; tisle, tislelizumab; nivo, nivolumab; ipi, ipilimumab; F1CDx, FoundationOne CDx; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; PFS, progression-free survival; TMB, tumor mutational burden; OS, overall survival.
Tumor Immune Microenvironment

The development of anticancer immunotherapies has benefited from advances in our knowledge of cancer immunity. The cancer-immune interaction represents a dynamic spatiotemporal process that involves multiple stimulatory factors and inhibitors acting at different stages of a cycle, as summarized by Chen and Mellman. ICls, the current mainstay of immunotherapies, aim to enhance killing of cancer cells by reactivating suppressed effector T cells in the TME. From this perspective, the TME can be generally classified into T-cell–inflamed versus non–T-cell–inflamed environments with the former being further classified on the basis of the activation status of the T cells. Notably, activated T-cell infiltration in the TME (T-cell–inflamed TME) is most often accompanied by an IFN-γ–driven adaptive immune resistance phenotype characterized by up-regulation of immune-regulatory pathways including immune-inhibitory receptors, such as PD-1, LAG-3, TIM-3, VISTA, and TIGIT, other inhibitory molecules, such as IDO-1, TGFβ1, and INOS, and or expansion of immune-inhibitory populations, such as regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs).

The presence of an abundant CD8+ cytotoxic T-cell infiltrate has been one of the most studied biomarkers, with multiple studies on the basis of IHC, mIF, or mRNA signatures. Unfortunately, each study has applied its own scoring method and cutoffs for high versus low CD8+ T cells, and patient populations were heterogeneous in size and characteristics. Evaluation of the activation state of the cytotoxic cells has also been proposed as an additional marker. For instance, co-expression of CD39, a marker of tumor-antigen-based activation, on CD8+ tumor-infiltrating T cells has been associated with up-regulation of both proliferation and T-cell dysfunction markers and increased response to PD-1 axis blockade. High expression of PD-1, another antigen-based activation and dysfunction marker, on CD8+ tumor-infiltrating T cells was predictive of both response and survival in NSCLC treated with anti–PD-1 ICls. Given that PD-1 axis blockade not only induces recovery of dysfunctional PD-1+ CD8+ T cells but also enhances PD-1+ Treg cell-mediated immunosuppression, a profound reactivation of effector PD-1+ CD8+ T cells rather than PD-1+ Treg cells may be necessary for tumor regression. The assessment of T-cell dysfunction could also include evaluation of a variety of markers including those for antigen-based activation (CD28, CD39, CD103, CD137, PD-1), regulation (checkpoint: PD-1, LAG-3, TIM-3), differentiation (CD45RO, Tbet, Eomes), proliferation (Ki-67), and apoptosis (Fas, Bim). Some of these markers assessed with mIF
have been found to have associations with survival after treatment with PD-1 or PD-L1 inhibitors in patients with NSCLC.\textsuperscript{44,68}

Among the multiple molecules and pathways, some may become predominant at a given time and thus considered as immune evasion “drivers.”\textsuperscript{71,72} For instance, elevated LAG-3 expression was found to be associated with either shorter\textsuperscript{66} or longer progression-free survival (PFS).\textsuperscript{62} The determinants for this apparent contradiction remain unknown, but multiple factors may be involved including the use of different cohorts, tissue imaging platforms applied, and marker cutoffs. In addition, the strong association of PD-L1 and LAG-3 expression observed in one of the studies\textsuperscript{62} suggested that the PD-1/PD-L1 pathway was the predominant “driver” immune-regulatory pathway. The possible role of the recently discovered LAG-3 ligand FGL1, known to be expressed in a fraction of NSCLCs, could be another determinant for these responses.\textsuperscript{73}

The local expansion of immune-suppressive populations in the TME, such as Treg, MDSC, and M2-polarized macrophages, in association with various immunosuppressive cytokines, such as interleukin-6, interleukin-8, and transforming growth factor-β is another mechanism for an adaptive immune resistance phenotype.\textsuperscript{69} Of those, MDSCs, a heterogeneous immature population of myeloid cells, contribute to resistance to ICIs by targeting effector T cells leading to T-cell dysfunction, promoting tumor angiogenesis, favoring an immunosuppressive network with M2 macrophage polarization, and Treg expansion.\textsuperscript{74}

The peripheral blood is also a potential source of biologically relevant information reflecting patient immune status. Circulating peripheral blood mononuclear cells assessed by flow cytometry can predict the efficacy of anti–PD-1 immunotherapy, with changes after treatment in percentage of various immune cells, including CD4+ T cells, CD8+ T cells, MDSCs, regulatory T cells, and PD-1–expressing T cells.\textsuperscript{75–77} In addition, soluble immune checkpoint-related proteins in the blood are associated with invasion and progression in NSCLC\textsuperscript{78} and harbor a predictive value in patients treated with immunotherapy.\textsuperscript{79–81}

**Transcriptomic Signatures**

Many transcriptomic signatures predictive of response or resistance to ICIs have been published in recent years (Table 4). These signatures are based on mRNA data obtained by targeted or whole transcriptome RNA-sequencing and retrieved from either public sources or specific studies. They have been validated on different cohorts and data sets and with different algorithms, including gene set enrichment analysis, to ascertain the gene function or to determine whether a predefined set of genes could be statistically relevant.

These predictive transcriptomic signatures consider sets of the genes involved in tumor antigenicity and T-cell priming and activation by interaction with activated dendritic cells, trafficking and infiltration of the T cells into tumors (CXCL9, CXCL10, CCL5 genes, among others), recognition of cancer cells by the T cells (HLA-A, HLA-B, HLA-C, B2M genes), infiltration by the inhibitory cells (such as MDSCs and Tregs) or molecules, and immune checkpoint receptor or ligand-encoding genes.

Many predictive signatures emphasize the role of T-cell inflammation and dysfunction including IFN-γ–related genes and T effector or immune cytolytic activities. Ayers et al.\textsuperscript{82} proposed, in a large cohort of pembrolizumab-treated patients across nine different tumor types, a T-cell–inflamed gene expression profiling consisting of 18 genes involved in T-cell–activated TME, IFN-γ signaling, antigen presentation, chemokines, T-cell cytotoxic activity, and adaptive immunity. This signature has been validated as an independent predictive biomarker of pembrolizumab monotherapy in a pan-cancer clinical trial performed across 20 tumor types, including SCLC (KEYNOTE-028).\textsuperscript{83,84} A high T-effector-IFN-γ–associated gene expression was also associated with improved outcomes with atezolizumab and durvalumab.\textsuperscript{85,86} Damotte et al.\textsuperscript{87} also reported a tumor inflammation signature associated with benefits from anti–PD-1 monotherapy. This signature was based on five genes related to IFN-γ signaling and antigen processing (CXCL9, CXCL10, CXCL11, TAP1, and PSMB9), and its expression was significantly higher in responders to PD-1 inhibitors. Similarly, macrophage M1 signature, peripheral T-cell signature, and high mRNA expression levels of CD137 and PSMB9 were more predictive of response to anti–PD-1 monotherapy than PD-L1 IHC, TMB, or tumor-infiltrating lymphocytes (TILs).\textsuperscript{88} Very recently, a 27-gene IO test score (IO score) independent of PD-L1 TPS and TMB helped in identifying patients benefiting from IO in the clinical setting.\textsuperscript{89}

Another group has investigated the predictive performance of DNA Damage Response (DDR) gene expression profiles along with markers of immune dysfunction and exclusion.\textsuperscript{90} Using a signature based on seven genes from eight DDR pathways, lung adenocarcinomas with low expression of DDR genes such as DUT, TYMS, and YWHAQ but high expression of MGMT, POLH, RAD1, and RAD17 harbored better survival. They were also considered more sensitive to ICIs on the basis of the Tumor Immune Dysfunction and Exclusion algorithm proposed by Jiang et al.\textsuperscript{91} These tumors also exhibited increased expressions of immune-inhibitory genes, such as TIM-3, ID01, LAG3, PD-L2, TIGIT, CD276, CD160, VEGFA, VEGFB, SLAMF7, KIR2DL3, and IL1B, and
## Table 4. Main Transcriptomic Signatures Predictive of Response or Resistance to ICIs in NSCLC

<table>
<thead>
<tr>
<th>Studies</th>
<th>Tumors (No. of Cases With RNAseq Data Available)</th>
<th>Platforms or Panels</th>
<th>Transcriptomic Signatures (GEP)</th>
<th>Pathways Involved</th>
<th>Clinical Validation p Values, AUC, and/or Hazard Ratios (95% Confidence Interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ott et al.83</td>
<td>20 cancers including SCLC (n = 8)</td>
<td>NanoString platform (custom 680-gene panel)</td>
<td>18-gene T-cell-inflamed GEP (CD3D, IDO1, CITA, CD3E, CCL5, GZMK, CD2, HLA-DR, CXCL13, IL2RG, NKG7, HLA-E, CXCR6, LAG3, TAGAP, CXCL10, STAT1, GZMB)</td>
<td>T-cell-activated TME, IFN-γ signaling, antigen presentation, chemokines, T-cell cytotoxic activity, and adaptive immunity</td>
<td>Higher scores associated with ORR (p = 0.012) and longer PFS (p = 0.017) in patients treated with pembrolizumab (KEYNOTE-028 trial)</td>
</tr>
<tr>
<td>Fehrenbacher et al.85</td>
<td>NSCLC (n = 224)</td>
<td>Fluidigm-based gene expression platform</td>
<td>8-Gene T-effector and IFN-γ GEP (CD8A, GZMA, GZMB, IFNγ, EOMES, CXCL9, CXCL10, and TBX21)</td>
<td>IFN-γ signaling</td>
<td>Higher Teff/IFN-γ scores associated improved OS (HR 0.43 [0.24–0.77]) vs. Teff/IFN-γ low (HR 1.10 [0.68–1.76]) in patients treated with atezolizumab (POPLAR trial)</td>
</tr>
<tr>
<td>Higgs et al.86</td>
<td>NSCLC (n = 97)</td>
<td>Pan-transcriptome sequencing</td>
<td>4-Gene IFN-γ GEP (IFNγ, CD274, LAG3, and CXCL9)</td>
<td>IFN-γ signaling</td>
<td>IFN-γ+ (vs. IFN-γ−) scores associated ORR 37.5 (21.7–56.3) vs. 6.2 (2.0–15.8); median OS: 22.7 mo (9.5–NR) vs. 6.5 (4.3–14.2); median PFS: 7.5 mo (2.7–14.6) vs. 1.4 (1.3–2.4) in patients treated with durvalumab</td>
</tr>
<tr>
<td>Damotte et al.87</td>
<td>NSCLC (n = 38)</td>
<td>NanoStringPanCancer IO 360-gene panel</td>
<td>5-Gene TIS (CXCL9, CXL10, CXCL11, TAP1, and PSMB9)</td>
<td>IFN-γ signaling and antigen processing</td>
<td>High TIS scores associated with improved OS (HR = 0.36 [0.14, 0.90], p = 0.02) in PD-1 inhibitor responders</td>
</tr>
<tr>
<td>Hwang et al.88</td>
<td>NSCLC (n = 21)</td>
<td>Oncomine Immune Response Research Assay (395 immune-related gene panel)</td>
<td>M1 (CBLB, CCR7, CD27, CD48, FOXO1, FYB, HLA-B, HLAG, IFIH1, IKZF4, LAMP3, NFKBIA, and SAMHD1) and peripheral T cell (HLA-DOA, GPR18, and STAT1) signatures</td>
<td>T-cell activation, antigen presentation, tumor-associated macrophages</td>
<td>Longer PFS associated with high M1 and peripheral T-cell GEP scores (p = 7.84e–5 and p = 8.29e–3) in patients treated with anti-PD-1 monotherapy. Positive predictive values (AUC) for peripheral T-cell signature: 0.94, IO score associated with DFS (HR 0.44 [0.21–0.97], p = 0.037)</td>
</tr>
<tr>
<td>Ranganath et al.89</td>
<td>NSCLC (n = 67)</td>
<td>In-house RT-qPCR panel</td>
<td>27-Gene IO score</td>
<td>DDR pathways</td>
<td>Low-risk score associated with better survival (HR 1.912 [1.421–2.573]; positive predictive values (AUC) 0.71</td>
</tr>
<tr>
<td>Leng et al.90</td>
<td>526 TCGA-LUAD and 438 LUAD cohort data sets (GSE30219, 3121, 50081)</td>
<td>Pan-transcriptome sequencing</td>
<td>7 Genes (DUT, TYMS, YWHAH, MGMT, POLH, RAD1, and RAD17) from 8 DDR GEPs</td>
<td>Good TIME (higher expression of immune-regulatory molecules, increased cytolytic activity, higher interferon-γ signature, and abundant immune cells) vs. bad TIME</td>
<td>“Good-TIME” associated with high response score to PD-1 inhibitors; positive predictive values (AUC) 0.702 (p = 0.039)</td>
</tr>
<tr>
<td>Jang et al.92</td>
<td>NSCLCs (n = 87 LUADs, 101 SCCs, and 35 NSCLCs for validation)</td>
<td>Pan-transcriptome sequencing and validation with the Nanostring platform (PanCancer Immune Profiling Panel of 770 mRNA sets)</td>
<td>59-Gene signature (IR score)</td>
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(continued)
decreased levels of B cells, CD8+ T cells, hematopoietic stem cells, and myeloid dendritic cells. Jang et al. have proposed a comprehensive signature of 59 genes from the Nanostring nCounter PanCancer Immune Profiling Panel of 770 mRNA set. Tumors with a “bad TiME” (Tumor immune Micro Environment) responsible for poor responses to ICIs were characterized by decreased expressions of MHC-I and MHC-II molecules, including HLA-A, -B, -C, and -DRB1, decreased tumor-associated dendritic cells and CD8+ T cells, an increased number of Tregs, and a low M1/M2 macrophage ratio. In contrast, tumors with a “good TiME,” indicating good response to anti–PD-1 treatment, were found to have up-regulation of PD-L1, PD-L2, CD8A, PD-1, CTLA-4, BTLA, CD40, CD40L, LAG3, TIGIT, TIM3, and VISTA. This good TiME also presented a high cytolytic activity and an increased IFN-γ signaling pathway expression. Memory B cells were also abundant, in contrast to Tregs. Interestingly, the predictive role of the B cells, complementary to total TILs, was also found with a B-cell expression signature, consisting of eight marker genes (BLK, CD19, FCRL2, MA4A1, TNFRSF17, TCL1A, SPIB, and PNOC), associated with response to ICIs.

New Technologies for Evaluation of TME

Given the plethora of immune-cell markers and cytokines involved in prediction of response or resistance to ICIs, multiplexed platforms have been widely used to simultaneously evaluate those predictive markers and determine the activation or dysfunction status of effector T cells, “driver” immune-inhibitory pathways, and extent of immune-inhibitory populations in the TME. Novel methods in development in immuno-oncology include the following: (1) liquid biopsy using multimodal cell-free or circulating tumor DNA (ctDNA) analysis; (2) highly multiplexed spatially resolved tissue analysis; and (3) high-dimensionality single-cell proteomic and or transcriptomic studies from cell suspensions. In general, these technologies require sophisticated instrumentation and relatively complex informatics for their analysis and interpretation. Therefore, they have been largely used in the research setting and for biomarker discovery. Nevertheless, the rapid advancement in their commercial availability, standardization, and progressive reduction in costs have allowed their incorporation into clinical trials.

The biomarker role of ctDNA in immuno-oncology has been extensively discussed elsewhere, and targeted ctDNA panels are currently used in the clinic for identification of gene mutations, measurement of TMB, and monitoring of disease response after treatment (e.g., minimal residual disease or assessment of “molecular response”). Newer modalities with biomarker potential
beyond mutation analysis include the assessment of ctDNA methylation marks and fragmentation patterns and identification of oncogenic viral sequences that seem to provide additional and complementary information.\textsuperscript{98–102}

The mIF enables simultaneous mapping of eight to 10 markers in one formalin-fixed, paraffin-embedded section. This technique remains limited compared with highly multiplexed spatially resolved technologies but seems more adapted to predictive marker validation in clinics, provided that they meet the guidelines of the Society for Immunotherapy of Cancer.\textsuperscript{103} In contrast, highly multiplexed, spatially resolved technologies are dedicated to biomarker discovery and can accommodate from 10 to 1000 markers, using a wide array of chemistries and signal detection systems. Examples include iterative cycling immunofluorescence protocols using primary fluorescence-conjugated antibodies and computational image alignment/integration (e.g., Cell DIVE/MultiOmyx\textsuperscript{104} and t-CyCIF\textsuperscript{105}); cycling protocols using antibodies conjugated to DNA barcodes for signal detection (e.g., CODEX\textsuperscript{106}); mass spectrometry-based analysis of metal-conjugated antibodies and focal high-energy tissue ionization (e.g., imaging mass cytometry\textsuperscript{107} and multiple ion beam imaging\textsuperscript{108}); localized mRNA or protein signal detection and counting using primary antibodies conjugated with photocleavable fluorescent DNA tags (e.g., digital spatial profiler/GeoMx\textsuperscript{109}) (Fig. 1); and spatial transcriptomics (sometimes also including proteins) using in situ mRNA hybridization and capture on tumor sections coupled to individual positional transcript barcoding and next-generation sequencing (e.g., VISIUM,\textsuperscript{110} SlideSeq,\textsuperscript{111}

**Figure 1.** Examples of DSP protein analysis. Selected ROIs for molecular quantification are revealed for a NSCLC tumor sample (pan-cytokeratin in green, CD45 in red). We can design ROIs containing only immune cells (ex ROI001), only tumor cells (not found), or regions that contain both immune cells and tumor cells (ex ROI004). In that case, an automatic segmentation, on the basis of the expression of panCK and CD45, allows the identification of two regions (in light green and purple, respectively, on the mixed ROI image) that will be analyzed independently. The selected proteins will be quantified in each region and the results used for differential expression analysis (among ROIs with different localization, different composition, among samples, etc.). DSP, digital spatial profiler; panCK, pancytokeratin; ROI, region of interest.
Dbitseq, and SeqScope). As an example, a recent analysis of 39-plex immune-related proteins on the GeoMx platform of 53 NSCLC tissue microarrays revealed that the level of stromal CD56-positive natural killer (NK)/NK T cells was significantly associated with better survival after PD-1 axis blockade. Another study that used a 37-plex imaging mass cytometry panel, including tumor- and immune-cell markers (Fig. 2), to evaluate 84 tissue microarrays of pretreatment NSCLCs revealed a prediction of durable clinical benefit with 97.3% mean accuracy and consistency using multidimensional markers and spatial patterns.

Highly dimensional, single-cell proteomic or transcriptomic studies from tumor cell extracts or liquid and fluid patient samples (e.g., blood and peripheral blood mononuclear cells, pleural effusion cells), although lacking spatial context, can capture numerous events. Multiple protein markers and single-cell phenotypes can be obtained using time-of-flight mass spectrometry (CyTOF platform) for simultaneous detection of approximately 30 to 40 metal-conjugated antibodies in fresh specimens. Recent studies using this platform coupled to mIF identified CD8+ effector T-cell exhaustion in human primary NSCLCs and revealed its association with reduced sensitivity to PD-1 axis blockade. Another study using a 32-marker CyTOF panel to study peripheral blood mononuclear cells from nine patients with advanced NSCLC treated with immunotherapy revealed an increased proportion of the NK cells associated with the treatment response. Single-cell RNA-sequencing detects the nuances in single-cell gene expression and enables to evaluate the specific cell subsets, pharmacodynamic changes, and intratumoral heterogeneity, in contrast to bulk-level transcriptomic, which provides averaged gene expression profiles of both tumor and TME populations. Individual single-cell RNA-sequencing platforms differ in their RNA capture methods, cDNA generation strategies, throughput, target coverage, and length of transcripts.

A recent study identified, in resectable NSCLC treated with neoadjuvant PD-1 ICI, tumor-antigen specific CD8+ T cells with tissue resident memory expression profiles, an incomplete effector program, increased immune-inhibitory signals, and expression of transcription factors associated with T-cell exhaustion. In preliminary explorations, T-cell exhaustion was associated with lack of benefit from the PD-1 axis blockade.

Another emerging technology that is worth mentioning here is artificial intelligence (AI) and machine learning. A recent study has revealed correlation of AI-powered spatial TIL assessment with tumor response and PFS in patients with advanced NSCLC treated with ICI suggesting its role as a complementary biomarker to PD-L1 IHC.

**Microbiome**

The microbiome is a dynamic collection of commensal organisms colonizing sites of the human

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**Figure 2.** (A) Representative images revealing a human FFPE NSCLC sample stained with multiplex immunofluorescence using DAPI, AE1-AE3 panCK, and CD3 with metal-conjugated antibodies (left panels) and subsequently analyzed using imaging mass cytometry (right panels). (B) Representative images from NSCLC stained simultaneously with a 37-marker IMC panel including tumor- and immune-cell markers. Selected markers are indicated within each caption. Bar = 100 μm. DAPI, 4',6-diamidino-2-phenylindole; FFPE, formalin-fixed, paraffin-embedded; IMC, imaging mass cytometry; panCK, pan-pancreatocytokeratin.
body and has been found to have an impact on anti-cancer immunity and response to immunotherapy.\textsuperscript{121} The commensal bacteria provide protection from pathogenic organisms and regulate host immunity by "crosstalk" with immune cells in the mucosa through pattern recognition receptors to a significant extent. The innate immune system is stimulated by the host microbiome that regulates local inflammation and helps to inform the adaptive immune response,\textsuperscript{122} although the exact mechanism by which altered microorganisms affect response to immunotherapy is unclear.

Several preclinical studies in mice have suggested that the gut microbiome plays a role in response to ICIs in solid tumors.\textsuperscript{123,124} Higher microbial diversity and higher proportions of "good" bacteria ("eubiosis") are associated with treatment response in patients with melanoma treated with PD-1 inhibitors.\textsuperscript{125,126} A landmark study by Routy et al.\textsuperscript{127} in 2018 revealed that patients with NSCLC with higher diversity of gut microbiome had improved response to ICI treatment. Akkermansia muciniphila and Enterococcus hirae were enriched in responders, and memory T-cell responses against A. muciniphila or E. hirae were found in patients with a better clinical response. Patients with NSCLC treated with antibiotics, known to alter the gut microbiome ("intestinal dysbiosis"), had reduced survival when given within 3 months of PD-1 blockade.\textsuperscript{127} Results of follow-up in vivo studies revealed fecal microbiota transplantation or oral supplementation with A. muciniphila overcame antibiotic-induced resistance to PD-1 blockade in mice.\textsuperscript{127} Small phase 1 clinical trials in patients with melanoma were found to have some benefit from fecal microbiome transplantation in overcoming ICI resistance.\textsuperscript{128,129} and feasibility studies of this approach are ongoing in NSCLC.\textsuperscript{130}

Other studies have revealed differences in gut microbiome between ICI responders and nonresponders in NSCLC with Firmicutes and higher microbial diversity associated with improved outcomes.\textsuperscript{131} In 37 Chinese patients with advanced NSCLC treated with nivolumab as part of CheckMate-078 and CheckMate-870, longer PFS was found in patients with high gut microbiome diversity.\textsuperscript{131} There were also differences in the microbiome composition between responders and nonresponders.\textsuperscript{131} In this study, previous antibiotic therapy did not affect outcome, but the study cohort was small. High microbiome diversity was associated with increased memory CD8+ T cells and NK cell signatures by peripheral blood flow cytometry.\textsuperscript{131}

Microbiome studies in lung cancer (and other solid tumors) have almost all focused on the gut microbiome; however, preclinical mouse studies have revealed that the lower respiratory tract microbiome affects local immunity and could potentially be more predictive than the gut microbiome for IO in lung cancer.\textsuperscript{132-134} An altered lung microbiome leading to a dysbiotic signature with increased oral commensals has been associated with tumor progression and poor prognosis in patients with NSCLC.\textsuperscript{132} Interestingly, modulation of the lung microbiome by aerosolized antibiotics has also been reported to promote immunity against lung metastasis in patients with melanoma.\textsuperscript{135}

Studies to date have focused on the diversity and abundance of the bacterial microbiome using metagenomic shotgun sequencing, 16S ribosomal RNA gene sequencing, and quantitative polymerase chain reaction techniques for selected bacteria in stool or respiratory tract samples.\textsuperscript{125,127} Nevertheless, the optimal method of evaluating the gut (or respiratory) microbiome has not been determined, and the potential impact of intrinsic and extrinsic factors such as race, diet, smoking, antibiotics, or other environmental exposures is poorly understood. Microbiome-wide association studies may provide more clarity in the future. Apart from bacteria, commensal fungi, viruses, and protozoans are integral components of the human microbiome that play an important role in tumor immunosurveillance.\textsuperscript{136,137}

Because the microbiome affects anticancer immune response with a "healthy" diverse microbiome favoring response to ICIs,\textsuperscript{125-127} the gut and lung microbiome could potentially be a predictive biomarker of response to IO, and its modulation may improve response to ICIs. More studies are needed to increase our understanding of the complex cancer-microbiome-immune axis and interplay between microbial ecology and host immunity to determine whether the microbiome could be a clinically useful biomarker.

**Major Pathologic Response to IO Neoadjuvant Therapy**

Major pathologic response (MPR) and complete pathologic response (CPR) have been used as an end point for a surrogate of clinical outcome in patients with lung cancer treated with various types of neoadjuvant therapy, including IO therapy. A number of studies have suggested that CPR after neoadjuvant chemotherapy may be a surrogate for overall survival.\textsuperscript{138-141} Nevertheless, it has been reported to be achieved in only 4% to 12% cases after chemotherapy making it a less optimal end point,\textsuperscript{142,143} whereas the recent clinical trial (CheckMate 816) reported a CPR rate of 30.5% after neoadjuvant therapy with nivolumab plus platinum-based chemotherapy.\textsuperscript{144} In contrast, MPR, defined as 10% or less of viable tumor cells at the primary tumor site in a surgically resected specimen, is more achievable; thus, there has been an increasing interest in its adoption. Although many studies revealed the prognostic
value of MPR, there are several technical issues in its assessment which have not been addressed until recently. Importantly, although MPR has been standardized across different types of specimens and therapies in other cancer types, such as breast, a standardized approach to postneoadjuvant surgically resected lung cancer specimens has until recently been lacking. The main questions include standardized approach to gross processing, histologic criteria of MPR, significance of different types of neoadjuvant therapy on histologic features of response (chemotherapy, targeted therapies, IO therapy), assessment of response in lymph nodes, and cutoff values for different histologic tumor types.

Currently, there have been two main attempts to address those issues including the IASLC multidisciplinary recommendation for pathologic assessment of lung cancer resection specimens after neoadjuvant therapy and pan-tumor pathologic scoring of response to PD-(L)1 blockade reported by Stein et al. The IASLC approach is specific for lung cancer and therapy agnostic, whereas the latter is tumor type agnostic but specific for immunotherapy, although the definitions of MPR and CPR are the same (10% or less and no residual viable tumor cells in the specimen, respectively). The question is whether the proposed scoring systems are interchangeable and applicable to different types of neoadjuvant therapies.

In postneoadjuvant therapy resections, proliferative fibrosis, neovascularization, cholesterol clefts, dense TILs, and tertiary lymphoid structures can be found in addition to reduction of viable tumor cells. These are considered to represent a state of immune activation in responders and, collectively, have been referred to as a “regression bed.” It is worth mentioning that these features can also be found in tumors treated with anti–PD-(L)1 combined with other agents such as chemotherapy or targeted therapies or chemotherapy alone, or, in a small proportion of cases, in tumors never exposed to neoadjuvant treatment. Importantly, the difference in interpreting stromal reaction is reflected to the difference in scoring % residual tumor volume between the above-mentioned two scoring systems. The pan-tumor pathologic scoring system includes intratumoral stroma as residual viable tumor, if features of “regression” are not present in immunotherapy-treated lung cancers, whereas the IASLC proposal considers viable tumor cells only as residual viable tumor. There are limited data to suggest that pathologists can consistently distinguish features of treatment response from preexisting stromal features.

To best of our knowledge, direct comparison studies between the two scoring systems have not been reported. Furthermore, although inter- and intraobserver concordance in scoring of postneoadjuvant surgically resected lung cancer specimens seems good after dedicated pathologist training and AI-powered digital assessment on pathology response seems to be strongly correlated with manual assessment, these need to be further confirmed.

Gross processing is the first critical step in the assessment of MPR. The above-mentioned approaches revealed some differences in the gross processing of postneoadjuvant lung cancer resection specimens, although complete sampling of a representative section of the tumor bed is the most often used approach.

Published studies in chemotherapy and chemoradiotherapy revealed prognostic significance of MPR with a cutoff of 10% or less viable tumor.

More recent studies suggested the best cutoff of 10% viable tumor for squamous cell carcinoma and that of 65% for adenocarcinoma. Preliminary data suggest that a cutoff of 10% or less viable tumor is a predictor of overall survival in patients treated with IO. It is possible that other cutoffs will emerge for lung cancer and perhaps for other tumor types as more data become available. Histologic features of response to IO are also emerging.

Histologic features of response found in the primary tumor can also be found in the lymph nodes. Nevertheless, the response to neoadjuvant treatment may be different between the primary tumor and lymph node metastases, and currently it is uncertain on how to define MPR in cases with minimal viable tumor at the primary site and substantial viable metastases. Recently published, retrospective studies of lung cancers treated with neoadjuvant chemotherapies revealed that nodal disease is a key determinant of outcomes and only patients with MPR and no residual nodal disease were found to have a survival benefit.

Currently, clinical responses are also evaluated radiologically using the Response Evaluation Criteria in Solid Tumors criteria as a standard method. Many studies on neoadjuvant chemotherapies revealed that a significant number of tumors with histologic CPR were radiologically evaluated as stable disease or partial response, and radiological response was not an independent prognostic factor. The similar, discrepant assessments have been reported in clinical trials with neoadjuvant nivolumab or nivolumab plus ipilimumab, with IO plus chemotherapy, or with a new IO agent.

The discordant results are likely attributed to the stromal immune activation and pose a further question as to which assessment is more representative for estimating the clinical response in the treatment using ICIs. Importantly, ctDNA clearance was associated with CPR in the CheckMate 816 clinical trial implying ctDNA analysis may serve as a predictive marker for neoadjuvant IO response.
Summary and Conclusion

Although there are a plethora of data on single diverse predictive biomarkers currently available in the IO space, head-to-head comparisons between multiple single biomarkers have been limited, and PD-L1 IHC remains the most often used predictive biomarker. Given the complex interaction between the immune system and tumor cells, multiple steps involved in the cancer immunity cycle and imperfect nature of individual biomarkers, a global assessment involving various biomarkers may be warranted to improve prediction of response to ICIs. Although some evidence suggests that a combination of biomarkers gives a better prediction of response and outcomes, the predictive performance of selected combinations needs to be tested and compared in prospective studies, ideally in clinical trials.

Furthermore, predictive biomarkers need to be practical. In that regard, new technologies may identify new biomarkers that can be easily implemented in clinical practice for a better adaptation of immunotherapies along with still incompletely understood mechanisms of primary and secondary resistance.

CRediT Authorship Contribution

Mari Mino-Kenudson: Conceptualization, Methodology, Resources, Writing (original), Writing (editing), Supervision.


Casey Connolly: Project management.

Sylvie Lantuejoul: Conceptualization, Methodology, Resources, Writing (original), Writing (editing), Supervision.

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References


51. Gainor JF, Shaw AT, Sequist LV, et al. EGFR mutations

60. Sanmamed MF, Eguren-Santamaria I, Schalper KA.

Overview of lung cancer immunotherapy. Cancer J

2020;26:473-484.


134. Dickson RP. The lung microbiome and ARDS. It is time to broaden the model. *Am J Respir Crit Care Med*. 2018;197:549-551.


