HHS Public Access

Author manuscript

Cancer. Author manuscript; available in PMC 2021 January 15.

Published in final edited form as:

Cancer. 2020 January 15; 126(2): 260–270. doi:10.1002/cncr.32468.

Biomarkers for immune checkpoint inhibition in non-small cell lung cancer (NSCLC)

J. Nicholas Bodor[1], **Yanis Boumber**[1,2,3], **Hossein Borghaei**[1,2]

[1]Department of Hematology / Oncology, Fox Chase Cancer Center, Philadelphia, PA

[2]Program in Molecular Therapeutics, Fox Chase Cancer Center, Philadelphia, PA

[3]Institute of Fundamental Medicine and Biology, Kazan Federal University, Kazan, Russia

Abstract

The emergence of immunotherapy has dramatically changed how NSCLC is treated and longer survival is now possible for some patients, even those with advanced disease. While some patients achieve durable responses to checkpoint blockade, not all experience such benefits, and some suffer from significant immunotoxicities. Given this, biomarkers that predict response to therapy are essential, and testing for tumor PD-L1 expression is the current standard. Extent of PD-L1 expression via immunohistochemisty (IHC) has demonstrated correlation with treatment response, though limitations with this marker exist. Recently, tumor mutational burden (TMB) has emerged as an alternative biomarker and studies have demonstrated its utility, irrespective of the PD-L1 level of a tumor. Gene expression signatures, tumor genotype, such as the presence of an oncogenic driver mutation, as well as density of tumor infiltrating lymphocytes (TILs) in the tumor microenvironment also seem to affect response to immunotherapy and are being researched. Peripheral serum markers are being studied and some have demonstrated to be predictive, though most are still investigational and need prospective validation. This paper reviews the biomarker PD-L1, as well as other emerging and investigational tissue-based and serum-based markers that have potential to better predict responders to immunotherapy.

Precis:

Immunotherapy has dramatically changed how advanced NSCLC is treated and longer survival is now possible for some patients. However, not all patients benefit from these agents and some suffer toxicities, highlighting the importance of biomarkers that predict efficacy. This article reviews several biomarkers including PD-L1, as well as other emerging and investigational tissue and serum-based markers that have potential to better predict responders to checkpoint inhibition.

Corresponding Author: Hossein Borghaei, DO, Professor of Medicine, Fox Chase Cancer Center, 333 Cottman Ave, Philadelphia PA 19111, hossein.borghaei@fccc.edu.

Conflict of Interest and Disclosures. Authors declare no conflict of interest. Y.B. has served on advisory boards of Astra Zeneca, AbbVie, Boehringer Ingelheim, Bristol-Meyers Squibb, Caris Life Sciences and Takeda. H. B. has received research support from Millennium, Merck, Celgene, BMS and Lilly and has served on advisory board for BMS, Lilly, Genentech, Celgene, Pfizer, Merck, EMD-Serono, Boehringer-Ingelheim, Astra Zeneca, Novartis, Genmab, Regeneron, BioNTech, Cantagria AB, Amgen, AbbVie and Axiom.

Keywords

Immunotherapy; Biomarkers; Non-Small Cell Lung Cancer; PD-L1; TMB

Introduction

Lung cancer is the leading cause of cancer-related death in the United States and worldwide. [1] The majority of these cancers are non-small cell lung cancer (NSCLC), of which the most common subtypes being adenocarcinoma and squamous cell carcinoma, and most patients have advanced disease on presentation.[2] Platinum-based chemotherapy has historically been the standard treatment for these patients, though responses to these agents are generally modest with relatively short intervals until disease progression.[3–6] More recently, immunotherapy has emerged as an exciting treatment alternative for patients without an actionable driver mutation, and has dramatically altered how advanced NSCLC is treated. [7,8]

An individual's immune system plays a key role in the monitoring and destruction of cancer cells, though this natural defense can be evaded by tumors cells and tolerance can develop via the upregulation of key immune checkpoints. Normally these checkpoints function to protect healthy tissue from infection-triggered cytotoxic immune responses, though tumor cells can take advantage of these same checkpoints to prevent immune-mediated destruction. For instance, the recognition of tumor antigens by T-cells results in the release of interferon-γ (INF-γ) which not only attracts other cytotoxic immune cells, but can also induce expression of the checkpoints that promote immune resistance, such as the programmed death ligand 1 (PD-L1) on tumor cells and indoleamine-2,3-dioxygenase (IDO) on dendritic cells or macrophages.[9,10] The cytotoxic T-lymphocyte associated antigen 4 (CTLA-4) is another important inhibitor of the immune response and serves to regulate early T-cell activation.[11,12] Immunotherapy agents work by blocking these checkpoint receptor-ligand interactions in a patient's immune system, leading to better activation of effector and cytotoxic T cells that respond to cancer cells.

The PD-1 inhibitors nivolumab and pembrolizumab, along with the PD-L1 inhibitor atezolizumab, have all demonstrated survival benefits over chemotherapy, and have all been approved for the treatment of metastatic NSCLC in the second-line.[13–15] Of these three agents, pembrolizumab is the only single-agent checkpoint inhibitor to be approved in the first-line setting, but only in patients with high PD-L1 expression.[16] Combining two modes of checkpoint blockade has also been investigated, and specifically the CTLA-4 inhibitor ipilimumab, with nivolumab has shown promise with results demonstrating enhanced antitumor activity with the combination, and increased progression free survival (PFS). Tumor mutation burden (TMB) has been highlighted as a potential important biomarker in the ipilimumab-nivolumab studies as patients with tumors with high mutational burden showing greater response to dual checkpoint inhibition with an objective response rate of 45% for the ipilimumab-nivolumab arm versus 27% for the chemotherapy arm. This benefit was seen across all PD-L1 level subgroups, including in tumors with expression levels less than 1%. [17,18] The recent findings from the MYSTIC trial which tested another dual immunotherapy combination, the CTLA-4 inhibitor tremelimumab with the PD-L1 inhibitor

durvalumab, did not find the combination to improve overall survival over chemotherapy in the study population, though in exploratory subgroup analyses a high blood-based TMB was associated with an increased overall survival time of 16.5 months for the tremelimumab – durvalumab group versus 10.5 months for the chemotherapy arm. [19]

Testing for PD-L1 expression remains the current standard for identifying NSCLC patients more likely to respond to immunotherapy. Recent chemo-immunotherapy trials have demonstrated benefit with the combination of a checkpoint inhibitor and platinum doublet chemotherapy for lung adenocarcinoma[20] and lung squamous cell carcinoma[21] for all levels of PD-L1, even in those with low expression. It is important to note though that less robust clinical outcomes were seen for the low PD-L1 expressing subgroups, thus emphasizing the continued relevance of PD-L1 as a biomarker even with chemo-immunotherapy use in the first-line. Despite the importance of PD-L1 expression as a predictor of response, many clinical trials testing single-agent checkpoint inhibitors have demonstrated that a subset of patients with high PD-L1 expression still do not benefit from these agents, thus highlighting some of its limitations.

Immunotherapy has resulted in significant benefits for many patients with metastatic NSCLC, though many still do not respond to therapy, have worsening disease on treatment, or suffer significant life-threatening immunotoxicities. [22–24] Nearly any organ in the body can be affected by an immune-related adverse event after checkpoint inhibitor therapy, though dermatitis, pneumonitis, colitis, and endocrinopathies tend to be most common. Cardiac, renal neurological, and hematological immune-related adverse events can also occur, though are rarer. While most cases are mild to moderate in severity, there are instances of severe and even fatal immune-related reactions, especially when not promptly recognized and appropriately managed.[25,26] For all these reasons, establishing biomarkers predictive of response to checkpoint inhibition is an essential step towards utilizing the new panel of immunotherapy agents in the most effective way. This paper reviews the currently approved biomarkers for immune checkpoint inhibition in NSCLC, and highlights emerging and investigational tissue and serum-based markers (Table).

PD-L1 Expression

Testing for PD-L1 expression by IHC, as mentioned, is the current standard to identify advanced NSCLC patients more likely to respond to immunotherapy in both treatment naïve patients and those who progress through standard chemotherapy. Multiple prospective trials have demonstrated correlation between level of tissue PD-L1 expression and clinical efficacy. In particular, KEYNOYE-024,[16] demonstrated superior overall survival for pembrolizumab compared with chemotherapy in patients with high PD-L1 expression, defined as PD-L1 expression on greater than half of tumor cells, i.e. PD-L1 ≥ 50%. This level of expression is now required to be eligible for treatment with pembrolizumab in treatment naïve patients with advanced NSCLC.

In second-line metastatic trials where patients were treated with either nivolumab or atezolizumab prolonged survival over docetaxel was demonstrated in all-comers, though greatest benefits were seen in tumors with higher PD-L1 expression.[13,15,27] The PACIFIC trial which included stage III patients with unresectable disease who completed definitive

chemotherapy with radiation, year-long consolidation therapy with durvalumab showed improved survival preferentially in patients with tumors with PD-L1 ≥ 25%, while no survival benefit was seen in patients with PD-L1 < 1%, in retrospective subgroup analyses.[28]

Despite the substantial evidence from these trials correlating levels of PD-L1 expression with response and clinical efficacy, PD-L1 remains a controversial biomarker of immunotherapy response and several issues limit its utility. Differences in testing platforms, use of varying cut-points for expression between different immunotherapy agents,[15,16] and the heterogenous nature of PD-L1 expression within tumors,[29,30] have all been points of criticism.[31–33]

A range of commercially available testing platforms exist, and each of the current FDA approved checkpoint inhibitors in NSCLC have a different PD-L1 IHC antibody used to assess expression. For instance, nivolumab uses a 28–8 antibody while pembrolizumab uses a 22C3 antibody clone. Atezolizumab's companion antibody clone is SP142 and durvalumab uses a SP263 antibody. Moreover, varying cut-points and scoring systems were used across trials for each of these agents to define levels of PD-L1 expression. Results from the phase III trials for nivolumab, pembrolizumab, and atezolizumab have led to FDA approvals with differing indications. Tumors with PD-L1 ≥ 50% qualify for the first-line single agent pembrolizumab, while tumors with PD-L1 ≥ 1% are eligible for pembrolizumab after progression on a platinum-based therapy per FDA approvals and the NCCN NSCLC guidelines.[34] On the other hand, nivolumab is approved in the second-line setting irrespective of PD-L1 expression. Unlike the 22C3, 28–8, and SP263 antibodies that assess PD-L1 expression just on tumor cell membranes, the SP142 antibody assay utilized by atezolizumab measures PD-L1 level on both tumor cells and infiltrating immune cells. Atezolizumab's scoring system results in four categories from least to greatest PD-L1 expression based on level of expression on tumor cells (TC) and immune cells (IC). The lowest category defined as TC0 and IC0 has PD-L1 < 1% on both TC and IC, while the highest category has PD-L1 ≥ 50% on TC or ≥ 10% on IC. Atezolizumab is approved in the second-line metastatic setting irrespective of PD-L1 expression, as all subgroups including the TC0 and IC0 (PD-L1 < 1%), demonstrated a survival benefit over docetaxel.[15]

Recent multi-center studies have attempted to compare the performance of each IHC PD-L1 assay and find commonalities between the companion tests. Notably, the Blueprint PD-L1 IHC Comparison Project, an industry-academic collaborative project, compared the four companion PD-L1 IHC assays for pembrolizumab (22C3), nivolumab (28–8), atezolizumab (SP142), and durvalumab (SP263). Results from Blueprint Phase 2, which was an expansion of the Phase 1 project, validated findings showing high comparable PD-L1 staining on tumor cells between the three companion antibody assays 22C3, 28–8, and SP263, while SP142 was found to be less sensitive.[35,36] In addition to the FDA approved IHC assays, laboratory developed tests (LDTs) for measurement of PD-L1 expression are utilized by oncology practices given the expense and low reimbursement rates associated with the FDA approved assays. While high comparability between the 22C3, 28–8, and SP263 antibody assays have been shown, recent analyses of LDTs have suggested variable concordance[37,38] and possible lower sensitivity.[39] Given the frequent use of these LDTs in practice, further study is required to carefully validate these assays.

Additionally, PD-L1 expression may be heterogeneous within tumors as seen in studies comparing expression between primary and metastatic sites. In particular, studies examining paired primary lung tumor tissue and metastatic brain tissue have demonstrated significant result disparities in PD-L1 expression.[29,30] The predictive value of PD-L1 may also be dependent on histological subtype. Correlation between PD-L1 expression and response to checkpoint blockade may be more relevant to adenocarcinoma rather than squamous cell. Checkmate 057, the phase III second-line nivolumab trial in advanced non-squamous tumors, found superior outcomes in patients with high PD-L1 expressing tumors.[13] Checkmate 017, the parallel trial in squamous cell lung cancer patients also demonstrated improved PFS and OS over docetaxel, though PD-L1 tumor expression was neither prognostic, nor predictive of response. [40]

While PD-L1 expression on the surface of tumor cells via IHC remains the recommended approach for predicting a tumor's responsiveness to immunotherapy, and testing is the current standard of care for any newly diagnosed NSCLC, it is a less than perfect predictor of response. Not all patients with high PD-L1 expression respond to therapy or demonstrate a durable clinical benefit, while some patients with low or no expression of PD-L1 derive benefit. In Keynote-024,[16] which only included patients with tumors with PD-L1≥ 50%, the overall response rate in the group treated with pembrolizumab was 45%, meaning that even in a population defined by their high levels of PD-L1 expression a significant proportion of patients did not exhibit any response to therapy. The limitations of PD-L1 as a biomarker has generated much interest in other potential markers of response, and most notably TMB has emerged as having potential predictive value and is further discussed in the next section.

Tumor Mutational Burden

Tumor mutational burden (TMB) may be a potential important biomarker of immunotherapy response and correlation between this marker and response rates of anti-PD-1 or anti-PD-L1 therapy have been demonstrated across several tumor types.[41] TMB is defined as the total number of mutations, including both base substitutions and short insertions/deletions, per coding area of a tumor genome. Previously whole exome sequencing was used to quantify the number of somatic mutations, though more recently next generation sequencing techniques using the FoundationOne CDx assay has been found to correlate closely and is now utilized to infer mutational burden. [17,42] The number of somatic mutations vary significantly between different cancer types, with melanoma having some of the highest number of mutations and gastrointestinal cancers, such as pancreatic cancer and mismatch repair proficient colorectal cancer, having some the lowest.[41] NSCLC spans a range in mutational burden, with a relative higher number of somatic mutations seen in smoking related lung cancer while never-smoking tumors exhibiting a lower burden. A high number of somatic mutations is thought to result in a greater number of neoantigens that are presented on the surface of tumor cells, which in turn increases immunogenicity and results in tumors becoming more sensitive to treatment with immune-checkpoint agents.[43]

This relationship between higher mutational burden and response to checkpoint inhibition has been demonstrated clinically in several studies. One of the early studies examining this was by Rizvi et al. who tested pembrolizumab in lung tumors with high non-synonymous

mutational and neoantigen levels, and they found that this was associated with longer PFS and improved durable clinical benefit.[43] Recent randomized trials, most notably Checkmate 227, investigated the utility of TMB in predicting response to the combination of ipilimumab with nivolumab.[17,18] This trial demonstrated superior PFS in patients with high TMB (defined as at least 10 mutation per megabase as determined by the FoundationOne CDx assay), irrespective of PD-L1 expression or histology, who received combination immunotherapy treatment instead of chemotherapy in the first-line metastatic setting (HR 0.58, 95% CI 0.41 – 0.81). This benefit was also seen in tumors with PD-L1 expression < 1%, but with high TMB. (HR 0.48, 95% CI 0.27 – 0.85). This latter result speaks to the imperfect predictive value of PD-L1 staining and demonstrates that the immunogenicity of a tumor may involve more than just level of PD-L1 expression. Despite these initial positive findings from Checkmate 227, the role of TMB as a biomarker for immunotherapy in NSCLC remains uncertain as subsequent overall survival data has revealed a statistically non-significant benefit of ipilimumab with nivolumab in high TMB patients (HR 0.77, 95% CI, 0.56–1.06), and moreover a comparable survival benefit was seen in patients with TMB < 10 mutations/megabase (HR 0.78; 95% CI, 0.61–1.00). In light of these data, the supplemental biologics license application seeking frontline FDA approval of ipilimumab with nivolumab for advanced NSCLC with TMB ≥10 mutations per megabase was withdrawn pending final data from Part 1a of Checkmate 227.[44] Moreover, TMB as a biomarker has other limitations including lack of standardization between the testing platforms used and the lack of an identified fixed TMB threshold defining a tumor as having “high” TMB. Varying thresholds of TMB have been used by different studies and while the recent ipilimumab with nivolumab trials initially suggested 10 mutations/megabase as being clinically meaningful, this marker was not predictive in the updated overall survival data as stated above.

Tumor Infiltrating Lymphocytes

The extent of lymphocyte infiltration seen within tumor tissue may have prognostic value as previous studies have correlated high levels of tumor infiltration lymphocytes (TILs) with improved survival across a range of cancer types,[45,46] including NSCLC.[47,48] Prior research in NSCLC has demonstrated that high levels of TILS, including CD8+, CD3+, and CD4+, is correlated with improved survival.[48] A high density of TILs is considered to reflect greater immune recognition of tumor cells in a patient, and represent a T-cell inflamed tumor microenvironment. This inflamed tumor phenotype may be more sensitive to checkpoint blockade and thus, in addition its prognostic role, TIL density has been studied for its predictive value as a biomarker for immunotherapy.

Studies in melanoma found that checkpoint blockade with ipilimumab [49] is associated with post-treatment increase in TILs, as well as clinical response. A study in patients with advanced melanoma treated with pembrolizumab, demonstrated an increase of intra-tumoral CD8+ T cells after treatment, which correlated with radiographic response. Intriguing data for NSCLC exist as well, with Herbst and his colleagues showing in a sample of fifty-three NSCLC cases an association between treatment response to atezolizumab and extent of PD-L1 expression on TILs ($P = 0.015$). [50] Interestingly, PD-L1 expression on tumor cells in this study was not correlated with response. The increase in the intra-tumoral presence of

infiltrative immune cells, both before and during treatment, may be predictive of clinical and radiographic response. Continued research to confirm this is needed, as well as determining clinically meaningful thresholds of increased TIL density.

Tumor Specific Genotypes

Testing for single-gene driver mutations, like EGFR and ALK, is the standard of care for any patient presenting with metastatic NSCLC. Despite the robust sustained responses many patients may have while on an oral targeted agent, like a tyrosine kinase inhibitor in EGFR exon 19 and 21 mutated tumors, [51,52] all patients will invariably progress on treatment or discontinue therapy due to toxicity. Immunotherapy is a treatment that has been utilized in such patients in future lines of therapy.

Unfortunately, EGFR and ALK positive tumors have not demonstrated the same responsiveness to checkpoint blockade as other genotypes,[53] and the second-line trials with single-agent immunotherapy have not demonstrated a survival benefit in this subgroup.[54,55] Hyperprogression after treatment with immunotherapy is also becoming a more widely recognized phenomenon in clinical practice[56] and there is some recent evidence to suggest EGFR tumor positivity is associated with hyperprogression, and that this may be mediated by the upregulation of PD-1 and PD-L1 seen with EGFR activation.[57] It is unclear whether this is a true phenomenon in EGFR mutated tumors though, as halting therapy with a TKI has the potential to lead to disease flare and may contribute to the worsening disease progression seen in some patients exposed to checkpoint inhibition.

Lack of responsiveness to single agent checkpoint inhibitors in EGFR positive tumors has been speculated to be due to lower levels of PD-L1 expression, though findings on this are inconsistent and requires further study.[53,55,58,59] More recently, Toki et al. studied 150 EGFR positive NSCLC tumor samples and found them to have lower levels of PD-L1 expression as well as a high density of “inactive” TILs, despite the presence of immune cells in the tumor microenvironment. TIL activity in this study was assessed by measuring levels of Ki67 and granzyme B. Inactive TILs had decreased levels of each of these markers reflecting low T-cell proliferation and low cytotoxic activity, respectively. The relative high frequency of inactive TILs in the EGFR positive tumor microenvironment may explain the relative lack of response seen in such tumors when challenged with immunotherapy. Contrary to this, in this same study KRAS positive tumors had higher levels of PD-L1 expression and greater density of “active” TILs.[59] Other studies have also demonstrated the increased immunogenicity of KRAS mutant tumors, [60] though it is important to note that KRAS mutant NSCLC overall is a heterogenous category with specific subgroups having varying responses to checkpoint inhibition. KRAS tumors with a co-mutation in STK11/LKB1, in particular has demonstrated primary resistance to PD-1 axis inhibitors in retrospective analyses. Tumors with a STK11/LKB1 genomic alteration treated with a checkpoint inhibitor demonstrated inferior clinical outcomes, including in tumors that were PD-L1 positive.[61]

While underlying factors for why certain tumor genotypes do not respond to checkpoint inhibition continue to be elucidated, recent trials utilizing combinations with immuno-chemotherapy and VEGF inhibition, suggest that such therapies may overcome the

resistance seen in EGFR and ALK positive tumors. Retrospective subgroup analyses from the clinical trial IMpower150 suggest that combining the checkpoint inhibitor atezolizumab with platinum doublet chemotherapy and the VEGF inhibitor bevacizumab can result in improved PFS over chemotherapy and bevacizumab alone in patients with EGFR or ALK mutant tumors (HR 0.59, 95% CI 0.37 – 0.94).[62] While such findings are encouraging and suggest the possibility of expanding the benefits of immunotherapy to tumor genotypes thought to be resistant, prospective validation is still needed.

Gene Expression Signatures

Gene expression profiling is an active area of research with studies suggesting its potential utility as a biomarker of checkpoint blockade. Immune gene signatures, in particular those associated with IFN-γ signaling and activated T-cells may have predictive value and have been correlated to immunotherapy response in several cancer types.[63–65] Ayers et al., demonstrated a IFN-γ related gene profile obtained from baseline tumor tissue was predictive of best overall response and PFS in cohorts of melanoma, head and neck cancer, and gastric cancer treated with pembrolizumab.[63] In the POPLAR trial, the phase II trial of atezolizumab in second-line treatment of advanced NSCLC, tumors with high expression of the T-effector and IFN-γ associated gene signature demonstrated improved survival (HR 0.43, 95% CI 0.24 – 0.77).[27] Interestingly, in IMpower150, the low expressors of the T-effector/INF-γ associated gene signature also demonstrated a PFS benefit with the atezolizumab with platinum doublet and bevacizumab treatment combination (HR 0.76, 95% CI 0.60–0.96), though higher expressors of this gene signature demonstrated a more robust benefit (HR 0.51, 95% CI 0.38–0.68).[62] Another recent study retrospectively examined the database of NSCLC patients from the original Rizvi et al., 2015 study[43] and via genomic tumor profiling found that the use of chemokine and immunosuppressive molecule expression profiles affirmed PD-L1 expression and improved the ability to predict response to pembrolizumab.[66] Multi-gene profiling holds promise for identifying immunogenic tumors more likely to benefit from immunotherapy agents, though further clinical study and prospective validation is needed.

Serum Based Biomarkers

Peripheral blood biomarkers are an attractive alternative to tumor-based markers, given the relative ease and less invasive nature of blood draws, as well as for the occasional but not uncommon instances of insufficient tissue sampling on some tumor biopsies. Laboratory values as collected during routine blood draws have been investigated for their potential as predictive markers of immunotherapy response. For instance, the neutrophil to lymphocyte ratio (NLR) has garnered much interest as a biomarker and can be easily calculated from the differential reported with a standard complete blood count. NLR is the ratio of the absolute neutrophil count to the absolute lymphocyte count. A tumor microenvironment with high neutrophil but low lymphocyte infiltration, is thought to promote greater angiogenesis and inhibit cell apoptosis, thus enhancing tumorigenesis and leading to poorer outcomes. Its utility has been studied in various cancer types, including melanoma, breast, and various gastrointestinal cancers.[67–70] NLR has been studied in NSCLC as well, and a high ratio at baseline may be a negative prognostic indicator in patients with metastatic lung cancer treated with immunotherapy.[71,72] In addition to being prognostic, NLR may serve as a

predictive marker and retrospective studies on nivolumab in the second-line setting have found that patient NLR correlates with treatment response[73] and may be helpful as an early marker of response.[74,75] Other blood count-based markers, such as absolute eosinophil count, absolute monocyte account, platelet to lymphocyte count (PLR), have also been studied with some correlating to response.[76,77] The NLR and other such peripheral blood markers may have utility in patients treated with checkpoint blockade, though studies thus far have been small and retrospective, and prospective validation is needed.

Blood-based tumor mutational burden (bTMB) is another investigational serum-based marker that has garnered interest. In a study by Gandara and his colleagues, the FoundationOne CDx assay was used to determine bTMB and a positive correlation between blood and tissue TMB was shown in a subset of samples obtained from the second-line atezolizumab trials, POPLAR and OAK (Spearman rank correlation = 0.64, 95% CI 0.56 – 0.71). Additionally in this same study, a retrospective analysis of data from these two trials found greater bTMB to be predictive of longer PFS in metastatic NSCLC patients treated with atezolizumab.[78] Their analyses suggest that bTMB ≥ 16 mutations/megabase may be a clinically meaningful and robust cut-point for defining “high” TMB. The MYSTIC trial,[19] which investigated the immunotherapy combination of tremelimumab with durvalumab in first-line stage IV NSCLC, also revealed the potential utility of bTMB as a biomarker. While MYSTIC was an overall negative study with the immunotherapy combination not improving survival over standard chemotherapy, exploratory analyses identified patients with bTMB ≥ 16 mutations/megabase and in this subgroup tremelimumab with durvalumab was associated with better overall survival as compared to chemotherapy (HR 0.62, 95% CI 0.45 – 0.86). In this study the GuardantOMNI assay was used to quantify mutational burden and a bTMB ≥ 16 mutations/megabase was found to correlate with a tissue TMB ≥ 10 mutations/megabase, as the latter was the cut-point utilized in Checkmate 227 that tested ipilimumab with nivolumab.[19]

Conclusion

Immunotherapy has ushered in an exciting time in the treatment of NSCLC and has dramatically changed how advanced disease is treated. Testing for PD-L1 expression via IHC as a biomarker for response remains the current standard of care and levels of expression has shown to be correlated with response in numerous studies. PD-L1 testing is not without limitations though, and not all patients with high expression will respond to immunotherapy, and some patients with low or no expression still respond. Such inconsistencies have prompted the investigation of other markers of response. TMB initially showed great promise, though its future role as a biomarker now remains uncertain given the updated overall survival data from Checkmate 227 suggesting lack of predictive value for ipilimumab with nivolumab. Specific tumor genotypes, such as EGFR and ALK, seem to be less responsive to checkpoint blockade, while some KRAS mutant tumors appear more sensitive to therapy. Other investigational tissue-based markers, like gene expression signatures, TIL concentration, or testing for “active” TILs show promise and studies have found these to correlate to immunotherapy response. Serum-based markers are an attractive option given the ease of obtaining such measures, especially when tissue sampling is insufficient for testing. Though still investigational, neutrophil to lymphocyte ratio (NLR),

bTMB, and other blood-based biomarkers have garnered interest both for their prognostic and predictive value when treating with checkpoint inhibitors.

In the last year, the chemo-immunotherapy combinations of pembrolizumab with a platinum doublet has become the new standard of care for first-line metastatic NSCLC with findings suggesting that such a combination can overcome the lack of efficacy previously seen in tumors with low levels of PD-L1 who received single agent immunotherapy. The results from Keynote 189 [20] which tested one such combination in advanced lung adenocarcinoma, led to the approval of this combination irrespective of PD-L1 expression as a survival benefit was seen over chemotherapy alone, even in patients with PD-L1 < 1%. It is important to note though, that in this subgroup of no PD-L1 expression, PFS was not significantly different between the combination therapy arm and the chemotherapy alone arm, and two-thirds of patients in this subgroup did not show an objective response. In addition, patients with EGFR and ALK positive tumors were excluded from this combination trial. The IMpower150 regimen of Atezolizumab with carboplatin and paclitaxel and the VEGF inhibitor bevacizumab was also recently approved by the FDA for first-line metastatic lung adenocarcinoma, though this approval did not include EGFR and ALK mutated patients. Atezolizumab combined with a platin and pemetrexed,[79] or carboplatin and nab-paclitaxel[80] has also been investigated and shown to improve clinical outcomes, though only the IMpower150 regimen is currently approved. Chemo-immunotherapy combinations will likely increase the number of patients who will be treated with a checkpoint inhibitor, though a proportion will still not benefit from a response. Moreover, treatment with checkpoint blockade is not without side effects or potential life-threatening immunotoxicities. This makes it all the more essential to continue the study and development of improved biomarkers that are predictors of toxicity, and sensitive and specific indicators of immunotherapy response.

Acknowledgments.

Y.B. was supported by the NIH R01 CA218802 and NIH R21 CA223394 grants, V Foundation translation award program T2018–013, and 2018–2019 Fox Chase Cancer Center Clinical Protocol Development Award. All authors are and in part supported by the NCI Core Grant, P30 CA006927, to Fox Chase Cancer Center.

References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward EFD. Global cancer statistics. Cancer J Clin 2011;61:69–90. doi:10.3322/caac.20107.
2. Lung Cancer (Non-small cell), American Cancer Society 2018 Available at: https://www.cancer.org/cancer/non-small-cell-lung-cancer.html.
3. Pujol JL, Breton JL, Gervais R, et al. Gemcitabine-docetaxel versus cisplatin-vinorelbine in advanced or metastatic non-small-cell lung cancer: A phase III study addressing the case for cisplatin. Ann Oncol 2005;16(4):602–610. doi:10.1093/annonc/mdi126. [PubMed: 15741225]
4. Sandler A, Gray R, Perry MC, et al. Paclitaxel–Carboplatin Alone or with Bevacizumab for Non–Small-Cell Lung Cancer. N Engl J Med 2006;355(24):2542–2550. doi:10.1056/NEJMoa061884. [PubMed: 17167137]
5. Schiller JH, Harrington D, Belani CP, et al. Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. N Engl J Med 2002;346(2):92–98. doi:10.1056/NEJMoa011954. [PubMed: 11784875]

6. Scagliotti G, Brodowicz T, Shepherd FA, et al. Treatment-by-histology interaction analyses in three phase III trials show superiority of pemetrexed in nonsquamous non-small cell lung cancer. J Thorac Oncol 2011;6(1):64–70. doi:10.1097/JTO.0b013e3181f7c6d4. [PubMed: 21119545]
7. Apetoh L, Ladoire S, Coukos G, Ghiringhelli F. Combining immunotherapy and anticancer agents: The right path to achieve cancer cure? Ann Oncol 2015;26(9):1813–1823. doi:10.1093/annonc/mdv209. [PubMed: 25922066]
8. Bodor JN, Kasireddy V, Borghaei H. First-Line Therapies for Metastatic Lung Adenocarcinoma Without a Driver Mutation. J Oncol Pract 2018;14(9):529–535. doi:10.1200/JOP.18.00250. [PubMed: 30205771]
9. Gajewski TF, Schreiber H, Fu YX. Innate and adaptive immune cells in the tumor microenvironment. Nat Immunol 2013;14(10):1014–1022. doi:10.1038/ni.2703. [PubMed: 24048123]
10. Spranger Stefani, Spaapen Robbert M., Zha Yuanyuan, et al. Up-Regulation of PD-L1, IDO, and Tregs in the Melanoma Tumor Microenvironment Is Driven by CD8+ T Cells. Sci Transl Med 2013;5(200):200ra116. doi:10.1126/scitranslmed.3006504.
11. Huang RR, Jalil J, Economou JS, et al. CTLA4 blockade induces frequent tumor infiltration by activated lymphocytes regardless of clinical responses in humans. Clin Cancer Res 2011;17(12):4101–4109. doi:10.1158/1078-0432.CCR-11-0407. [PubMed: 21558401]
12. Chambers C, Kuhns M, Egen J. CTLA-4-mediated inhibition in regulation of T cell responses: mechanisms and manipulation in tumor immunotherapy. Annu Rev Immunol 2001;19:565–594.. [PubMed: 11244047]
13. Borghaei H, Paz-Ares L, Horn L, et al. Nivolumab versus Docetaxel in Advanced Nonsquamous Non-Small-Cell Lung Cancer. N Engl J Med 2015;373(17):1627–1639. doi:10.1056/NEJMoa1507643. [PubMed: 26412456]
14. Herbst RS, Baas P, Kim DW, et al. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): A randomised controlled trial. Lancet 2016;387(10027):1540–1550. doi:10.1016/S0140-6736(15)01281-7. [PubMed: 26712084]
15. Rittmeyer A, Barlesi F, Waterkamp D, et al. Atezolizumab versus docetaxel in patients with previously treated non-small-cell lung cancer (OAK): a phase 3, open-label, multicentre randomised controlled trial. Lancet 2017;389(10066):255–265. doi:10.1016/S0140-6736(16)32517-X. [PubMed: 27979383]
16. Reck M, Rodríguez-Abreu D, Robinson AG, et al. Pembrolizumab versus Chemotherapy for PD-L1–Positive Non–Small-Cell Lung Cancer. N Engl J Med 2016;375(19):1823–1833. doi:10.1056/NEJMoa1606774. [PubMed: 27718847]
17. Hellmann MD, Ciuleanu T-E, Pluzanski A, et al. Nivolumab plus Ipilimumab in Lung Cancer with a High Tumor Mutational Burden. N Engl J Med 2018;378:2093–2104. doi:10.1056/NEJMoa1801946. [PubMed: 29658845]
18. Hellmann MD, Rizvi NA, Goldman JW, et al. Nivolumab plus ipilimumab as first-line treatment for advanced non-small-cell lung cancer (CheckMate 012): results of an open-label, phase 1, multicohort study. Lancet Oncol 2017;18(1):31–41. doi:10.1016/S1470-2045(16)30624-6. [PubMed: 27932067]
19. Rizvi NA, Cho BC, Niels R, Lee KH, Al E. Durvalumab with or without tremelimumab vs platinum-based chemotherapy as first-line treatment for metastatic non-small cell lung cancer: MYSTIC. Presented at 2018 European Society of Medical Oncology In: ; 2018.
20. Gandhi L, Rodríguez-Abreu D, Gadgeel S et al. Pembrolizumab plus Chemotherapy in Metastatic Non-Small-Cell Lung Cancer. N Engl J Med 2018;378:2078–2092. doi:doi: 10.1056/NEJMoa1801005. [PubMed: 29658856]
21. Paz-Ares L, Luft A, Vicente D, et al. Pembrolizumab plus Chemotherapy for Squamous Non–Small-Cell Lung Cancer. N Engl J Med 2018;379:2040–2051. doi:10.1056/NEJMoa1810865. [PubMed: 30280635]
22. Kumar V, Chaudhary N, Garg M, Floudas CS, Soni P, Chandra AB. Current Diagnosis and Management of Immune Related Adverse Events (irAEs) Induced by Immune Checkpoint Inhibitor Therapy. Front Pharmacol 2017;8(MAY). doi:10.3389/fphar.2017.00311.

23. Bertrand A, Kostine M, Barnetche T, Truchetet M-E, Schaeverbeke T. Immune related adverse events associated with anti-CTLA-4 antibodies: systematic review and meta-analysis. BMC Med 2015;13(1):211. doi:10.1186/s12916-015-0455-8. [PubMed: 26337719]
24. Naidoo J, Wang X, Woo KM, et al. Pneumonitis in patients treated with anti-programmed death-1/programmed death ligand 1 therapy. J Clin Oncol 2017;35(7):709–717. doi:10.1200/JCO.2016.68.2005. [PubMed: 27646942]
25. Puzanov I, Diab A, Abdallah K, et al. Managing toxicities associated with immune checkpoint inhibitors: Consensus recommendations from the Society for Immunotherapy of Cancer (SITC) Toxicity Management Working Group. J Immunother Cancer. 2017;5(1). doi:10.1186/s40425-017-0300-z.
26. Winer A, Bodor JN, Borghaei H. Identifying and managing the adverse effects of immune checkpoint blockade. J Thorac Dis 2018;10:S480–S489. doi:10.21037/jtd.2018.01.111. [PubMed: 29593893]
27. Fehrenbacher L, Spira A, Ballinger M, et al. Atezolizumab versus docetaxel for patients with previously treated non-small-cell lung cancer (POPLAR): A multicentre, open-label, phase 2 randomised controlled trial. Lancet 2016;387(10030):1837–1846. doi:10.1016/S0140-6736(16)00587-0. [PubMed: 26970723]
28. Antonia SJ, Villegas A, Daniel D, et al. Overall Survival with Durvalumab after Chemoradiotherapy in Stage III NSCLC. N Engl J Med 2018;379:2342–2350. doi:10.1056/NEJMoa1809697. [PubMed: 30280658]
29. Mansfield AS, Aubry MC, Moser JC, et al. Temporal and spatial discordance of programmed cell death-ligand 1 expression and lymphocyte tumor infiltration between paired primary lesions and brain metastases in lung cancer. Ann Oncol 2016;27(1):1953–1958. doi:10.1093/annonc/mdw289. [PubMed: 27502709]
30. Zhou J, Gong Z, Jia Q, Wu Y, Yang ZZ, Zhu B. Programmed death ligand 1 expression and CD8+ tumor-infiltrating lymphocyte density differences between paired primary and brain metastatic lesions in non-small cell lung cancer. Biochem Biophys Res Commun 2018;498(4):751–757. doi:10.1016/j.bbrc.2018.03.053. [PubMed: 29526752]
31. Gridelli C, Ardizzoni A, Barberis M, et al. Predictive biomarkers of immunotherapy for non-small cell lung cancer: Results from an Experts Panel Meeting of the Italian Association of Thoracic Oncology. Transl Lung Cancer Res 2017;6(3):373–386. doi:10.21037/tlcr.2017.05.09. [PubMed: 28713682]
32. Gibney GT, Weiner LM, Atkins MB. Predictive biomarkers for checkpoint inhibitor-based immunotherapy. Lancet Oncol 2016;17(12):e542–e551. doi:10.1016/S1470-2045(16)30406-5. [PubMed: 27924752]
33. Voong KR, Feliciano J, Becker D, Levy B. Beyond PD-L1 testing-emerging biomarkers for immunotherapy in non-small cell lung cancer. Ann Transl Med 2017;5(18):376–376. doi:10.21037/atm.2017.06.48. [PubMed: 29057236]
34. NCCN Guidelines. https://www.nccn.org/professionals/physician_gls/default.aspx. Published 2018.
35. Hirsch FR, McElhinny A, Stanforth D, et al. PD-L1 Immunohistochemistry Assays for Lung Cancer: Results from Phase 1 of the Blueprint PD-L1 IHC Assay Comparison Project. J Thorac Oncol 2017;12(2):208–222. doi:10.1016/j.jtho.2016.11.2228. [PubMed: 27913228]
36. Tsao MS, Kerr KM, Kockx M, et al. PD-L1 Immunohistochemistry Comparability Study in Real-Life Clinical Samples: Results of Blueprint Phase 2 Project. J Thorac Oncol 2018;13(9):1302–1311. doi:10.1016/j.jtho.2018.05.013. [PubMed: 29800747]
37. Adam J, Le Stang N, Rouquette I, et al. Multicenter harmonization study for PD-L1 IHC testing in non-small-cell lung cancer. Ann Oncol 2018;29(4):953–958. doi:10.1093/annonc/mdy014. [PubMed: 29351573]
38. Rimm DL, Han G, Taube JM, et al. A prospective, multi-institutional, pathologist-based assessment of 4 immunohistochemistry assays for PD-L1 expression in non–small cell lung cancer. JAMA Oncol 2017;3(8):1051–1058. doi:10.1001/jamaoncol.2017.0013. [PubMed: 28278348]

39. Velcheti V, Patwardhan PD, Liu FX, Chen X, Cao X, Burke T. Real-world PD-L1 testing and distribution of PD-L1 tumor expression by immunohistochemistry assay type among patients with metastatic non-small cell lung cancer in the United States. PLoS One. 2018;13(11). doi:10.1371/journal.pone.0206370.
40. Brahmer J, Reckamp K, Baas P, et al. Nivolumab versus docetaxel in advanced squamous-cell non-small-cell lung cancer. N Engl J Med 2015;373(2):123–135. doi:10.1056/NEJMoa1504627LK - [PubMed: 26028407]
41. Yarchoan M, Hopkins A, Jaffee EM. Tumor Mutational Burden and Response Rate to PD-1 Inhibition. N Engl J Med 2017;377(25):2500–2501. doi:10.1056/NEJMc1713444. [PubMed: 29262275]
42. FoundationOne CDx. www.foundationmedicine.com/genomic-testing/foundation-one-cdx.
43. Rizvi NA, Hellmann MD, Snyder A, et al. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. Science (80-). 2015;348(6230):124–128. doi:10.1126/science.aaa1348.
44. Bristol-Myers Squibb Reports Fourth Quarter and Full Year Financial Results. Bristol-Myers Squibb. Published 1 24, 2019.
45. Schatton T, Scolyer RA, Thompson JF, Mihm MC. Tumor-infiltrating lymphocytes and their significance in melanoma prognosis. Methods Mol Biol 2014;1102:287–324. doi:10.1007/978-1-62703-727-3_16. [PubMed: 24258985]
46. Thomas NE, Busam KJ, From L, et al. Tumor-infiltrating lymphocyte grade in primary melanomas is independently associated with melanoma-specific survival in the population-based genes, environment and melanoma study. J Clin Oncol 2013;31(33):4252–4259. doi:10.1200/JCO.2013.51.3002. [PubMed: 24127443]
47. Brambilla E, Le Teuff G, Marguet S, et al. Prognostic effect of tumor lymphocytic infiltration in resectable non-small-cell lung cancer. J Clin Oncol 2016;34(11):1223–1230. doi:10.1200/JCO.2015.63.0970. [PubMed: 26834066]
48. Zeng D-Q, Yu Y-F, Ou Q-Y, et al. Prognostic and predictive value of tumor-infiltrating lymphocytes for clinical therapeutic research in patients with non-small cell lung cancer. Oncotarget. 2016;7(12):13765–13781. doi:10.18632/oncotarget.7282.
49. Hamid O, Schmidt H, Nissan A, et al. A prospective phase II trial exploring the association between tumor microenvironment biomarkers and clinical activity of ipilimumab in advanced melanoma. J Transl Med 2011;9:204. doi:10.1186/1479-5876-9-204. [PubMed: 22123319]
50. Herbst R, Soria J-C, Kowanetz M, et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. Nature. 2014;515(7528):563–567. doi:10.1038/nature14011. [PubMed: 25428504]
51. Soria J-C, Ohe Y, Vansteenkiste J, et al. Osimertinib in Untreated EGFR-Mutated Advanced Non–Small-Cell Lung Cancer. N Engl J Med 2017;378:113–125. doi:10.1056/NEJMoa1713137. [PubMed: 29151359]
52. Rosell R, Carcereny E, Gervais R, et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): A multicentre, open-label, randomised phase 3 trial. Lancet Oncol 2012;13(3):239–246. doi:10.1016/S1470-2045(11)70393-X. [PubMed: 22285168]
53. Gainor JF, Shaw AT, Sequist LV., et al. EGFR mutations and ALK rearrangements are associated with low response rates to PD-1 pathway blockade in non-small cell lung cancer: A retrospective analysis. Clin Cancer Res 2016;22(18):4585–4593. doi:10.1158/1078-0432.CCR-15-3101. [PubMed: 27225694]
54. Lee CK, Man J, Lord S, et al. Checkpoint Inhibitors in Metastatic EGFR-Mutated Non–Small Cell Lung Cancer—A Meta-Analysis. J Thorac Oncol 2017;12(2):403–407. doi:10.1016/j.jtho.2016.10.007. [PubMed: 27765535]
55. Bylicki O, Paleiron N, Margery J, et al. Targeting the PD-1/PD-L1 Immune Checkpoint in EGFR-Mutated or ALK-Translocated Non-Small-Cell Lung Cancer. Target Oncol 2017;12(5):563–569. doi:10.1007/s11523-017-0510-9. [PubMed: 28624922]

56. Champiat S, Dercle L, Ammari S, et al. Hyperprogressive disease is a new pattern of progression in cancer patients treated by anti-PD-1/PD-L1. Clin Cancer Res 2017;23(8):1920–1928. doi:10.1158/1078-0432.CCR-16-1741. [PubMed: 27827313]
57. Kato S, Goodman A, Walavalkar V, Barkauskas DA, Sharabi A, Kurzrock R. Hyperprogressors after immunotherapy: Analysis of genomic alterations associated with accelerated growth rate. Clin Cancer Res 2017;23(15):4242–4250. doi:10.1158/1078-0432.CCR-16-3133. [PubMed: 28351930]
58. Vokes N, Jimenez Alguilar E, Adeni A, Al E. Efficacy and Genomic Correlates of Response to Anti-PD1/PD-L1 Blockade in Non-Small Cell Lung Cancers Harboring Targetable Oncogenes. 2018 IASLC World Conference on Lung Cancer.
59. Toki MI, Mani N, Smithy JW, et al. Immune Marker Profiling and Programmed Death Ligand 1 Expression Across NSCLC Mutations. J Thorac Oncol 2018;13(12):1884–1896. doi:10.1016/j.jtho.2018.09.012. [PubMed: 30267840]
60. Dong ZY, Zhong WZ, Zhang XC, et al. Potential predictive value of TP53 and KRAS mutation status for response to PD-1 blockade immunotherapy in lung adenocarcinoma. Clin Cancer Res 2017;23(12):3012–3024. doi:10.1158/1078-0432.CCR-16-2554. [PubMed: 28039262]
61. Skoulidis F, Goldberg ME, Greenawalt DM, et al. STK11/LKB1 mutations and PD-1 inhibitor resistance in KRAS-mutant lung adenocarcinoma. Cancer Discov 2018;8(7):822–835. doi:10.1158/2159-8290.CD-18-0099. [PubMed: 29773717]
62. Socinski MA, Jotte RM, Cappuzzo F, et al. Atezolizumab for First-Line Treatment of Metastatic Nonsquamous NSCLC. N Engl J Med 2018;378(24):2288–2301. doi:10.1056/NEJMoa1716948. [PubMed: 29863955]
63. Ayers M, Lunceford J, Nebozhyn M, et al. IFN-γ-related mRNA profile predicts clinical response to PD-1 blockade. J Clin Invest 2017;127(8):2930–2940. doi:10.1172/JCI91190. [PubMed: 28650338]
64. Prat A, Navarro A, Paré L, et al. Immune-related gene expression profiling after PD-1 blockade in non–small cell lung carcinoma, head and neck squamous cell carcinoma, and melanoma. Cancer Res 2017;77(13):3540–3550. doi:10.1158/0008-5472.CAN-16-3556. [PubMed: 28487385]
65. Ji RR, Chasalow SD, Wang L, et al. An immune-active tumor microenvironment favors clinical response to ipilimumab. Cancer Immunol Immunother 2012;61(7):1019–1031. doi:10.1007/s00262-011-1172-6. [PubMed: 22146893]
66. Brogden KA, Parashar D, Hallier AR, et al. Genomics of NSCLC patients both affirm PD-L1 expression and predict their clinical responses to anti-PD-1 immunotherapy. BMC Cancer. 2018;18(1). doi:10.1186/s12885-018-4134-y.
67. Liu X, Qu JK, Zhang J, et al. Prognostic role of pretreatment neutrophil to lymphocyte ratio in breast cancer patients. Med (United States). 2017;96(45):e8101. doi:10.1097/MD.0000000000008101.
68. Koh C, Bhoo-Pathy N, Ng K, et al. Elevated neutrophil lymphocyte ratio predicts survival in breast cancer. Cancer Res 2015;75(9). doi:10.1158/1538-7445.SABCS14-P6-08-32.
69. Szor D, Dias A, Pereira M, et al. Prognostic Role of Neutrophil/Lymphocyte Ratio in Resected Gastric Cancer: A Systematic Review and Meta-analysis. Clinics. 2018;73:e360. doi:10.6061/clinics/2018/e360.
70. Mei Z, Shi L, Wang B, et al. Prognostic role of pretreatment blood neutrophil-to-lymphocyte ratio in advanced cancer survivors: A systematic review and meta-analysis of 66 cohort studies. Cancer Treat Rev 2017;58:1–13. [PubMed: 28602879]
71. Diem S, Schmid S, Krapf M, et al. Neutrophil-to-Lymphocyte ratio (NLR) and Platelet-to-Lymphocyte ratio (PLR) as prognostic markers in patients with non-small cell lung cancer (NSCLC) treated with nivolumab. Lung Cancer. 2017;111:176–181. doi:10.1016/j.lungcan.2017.07.024. [PubMed: 28838390]
72. Cedres S, Torrejon D, Martinez A, et al. Neutrophil to lymphocyte ratio (NLR) as an indicator of poor prognosis in stage IV non-small cell lung cancer. Clin Transl Oncol 2012;14(11):864–869. [PubMed: 22855161]

73. Kiriu T, Yamamoto M, Nagano T, et al. The time-series behavior of neutrophil-to-lymphocyte ratio is useful as a predictive marker in non-small cell lung cancer. PLoS One. 2018;13(2):e0193018. doi:10.1371/journal.pone.0193018.
74. Takeda T, Takeuchi M, Saitoh M, Takeda S. Neutrophil-to-lymphocyte ratio after four weeks of nivolumab administration as a predictive marker in patients with pretreated non-small-cell lung cancer. Thorac Cancer. 2018;9(10):1291–1299. doi:10.1111/1759-7714.12838. [PubMed: 30126063]
75. Nakaya A, Kurata T, Yoshioka H, et al. Neutrophil-to-lymphocyte ratio as an early marker of outcomes in patients with advanced non-small-cell lung cancer treated with nivolumab. Int J Clin Oncol 2018;23(4):634–640. doi:10.1007/s10147-018-1250-2. [PubMed: 29442281]
76. Soyano A, Dholaria B, Marin J, et al. Blood biomarkers correlate with outcome in advanced non-small cell lung cancer patients treated with anti PD-1 antibodies. J Thorac Oncol 2017;12(11):S2011-S2011..
77. Tanizaki J, Haratani K, Hayashi H, et al. Peripheral Blood Biomarkers Associated with Clinical Outcome in Non–Small Cell Lung Cancer Patients Treated with Nivolumab. J Thorac Oncol 2018;13(1):97–105. doi:10.1016/j.jtho.2017.10.030. [PubMed: 29170120]
78. Gandara DR, Paul SM, Kowanetz M, et al. Blood-based tumor mutational burden as a predictor of clinical benefit in non-small-cell lung cancer patients treated with atezolizumab. Nat Med 2018;24(9):1441–1448. doi:10.1038/s41591-018-0134-3. [PubMed: 30082870]
79. Papadimitrakopoulou V, Cobo M, Bordoni R, et al.: Impower132: PFS and safety results with 1L atezolizumab + carboplatin/cisplatin + pemetrexed in stage IV non-squamous NSCLC. IASLC World Conference on Lung Cancer Abstract OA05.07. Presented 9 2018.
80. Cappuzzo F, McCleod M, Hussein M, et al., IMpower130: Progression-free survival (PFS) and safety analysis from a randomised phase III study of carboplatin + nab-paclitaxel (CnP) with or without atezolizumab (atezo) as first-line (1L) therapy in advanced non-squamous NSCLC, Annals of Oncology, Volume 29, Issue suppl_8, 10 2018

Table.

Tissue and serum-based biomarkers for immunotherapy (IO) response in non-small cell lung cancer

Biomarker of Interest	Assay Details	Outcomes/Literature Support
Tissue-based		
PD-L1 Expression	Immunohistochemisty (IHC) to determine proportion of PD-L1 positivity/expression.	Greater PD-L1 positivity/expression associated with improved outcomes in first-line and second-line advanced NSCLC trials using IO.[13–15]
Tumor Mutational Burden (TMB)	Whole exome sequencing or FoundationOne CDx assay to quantify the number of somatic mutations per coding area of a tumor genome.	Higher TMB associated with improved PFS, though not OS, with first-line ipilimumab/nivolumab in advanced NSCLC, irrespective of PD-L1 expression. [17]
Tumor Infiltrating Lymphocytes (TILs)	Assessment of lymphocyte infiltration seen within tumor tissue.	Higher TIL density associated with improved survival in NSCLC.[47,48] Extent of PD-L1 expression on TILs associated with response to atezolizumab. [50]
Tumor Specific Genotypes	Fluorescence in situ hybridization (FISH) or next generation sequencing to identify genomic alterations in EGFR, ALK, KRAS etc.	EGFR and ALK mutated tumors associated with poorer outcomes in second-line IO trials. [54] STK11/LKB1 co-mutation associated with IO resistance.[61]
Gene Expression Signatures	Multi-gene profiling to identify immunogenic gene signatures, e.g. activated T-cell, IFN-y	High expression of T-effector and INF-y related gene signature associated with improved OS with second-line atezolizumab in advanced NSCLC.[27]
Serum-based		
Complete Blood Count (CBC) Markers (NLR, PLR, etc.)	Neutrophil to lymphocyte ratio (NLR), platelet to lymphocyte ratio (PLR), absolute eosinophil count, and others as calculated from CBC differential.	Higher NLR associated with poorer prognosis in advanced NSCLC.[71,72] NLR correlated to treatment response in second-line nivolumab studies.[74,75]
Blood Tumor Mutational Burden (bTMB)	FoundationOne CDx with quantification of single nucleotide variants, GuardantOMNI CDx assay.	Higher bTMB associated with longer PFS with second-line atezolizumab in advanced NSCLC.[78] High bTMB subgroup with improved OS with first-line tremelimumab/durvulumab. [19]

IO - immunotherapy