Review

Clinical Cancer Research

Immunotherapy in Non–Small Cell Lung Cancer: Facts and Hopes

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Abstract

Immune-checkpoint inhibitors (ICI), particularly inhibitors of the PD-1 axis, have altered the management of non–small cell lung cancer (NSCLC) over the last 10 years. First demonstrated to improve outcomes in second-line or later therapy of advanced disease, ICIs were shown to improve overall survival compared with chemotherapy in first-line therapy for patients whose tumors express PD-L1 on at least 50% of cells. More recently, combining ICIs with chemotherapy has been shown to improve survival in patients with both squamous and nonsquamous NSCLC, regardless of PD-L1 expression. However, PD-L1 and, more recently, tumor mutational burden have not proven to be straightforward indicative biomarkers. We describe the advances to date in utilizing these biomarkers, as well as novel markers of tumor inflammation, to ascertain which patients are most likely to benefit from ICIs. Ongoing translational work promises to improve the proportion of patients who benefit from these agents.

Introduction

In the last 10 years, the introduction of immune-checkpoint inhibitors (ICI) into the treatment of non–small cell lung cancer (NSCLC) has transformed the therapeutic landscape in this recalcitrant disease. And yet the development of ICIs in lung cancer has taken a somewhat unexpected path. In this *Facts and Hopes*, we describe the progress made to date, our understanding of how biomarkers might be used to identify patients most likely to respond to ICIs, and suggest how our growing understanding of primary and acquired resistance might be used to improve outcomes in what are truly a complex, interrelated set of diseases.

Checkpoint Inhibitors in the Metastatic Setting

Large randomized clinical trials of monoclonal antibodies against programmed death ligand-1 (PD-1) first demonstrated significant antitumor activity in the heavily pretreated metastatic setting in 2015, when the PD-1 inhibitor nivolumab was shown to produce improvements in overall survival (OS) compared with second-line docetaxel in both advanced squamous and nonsquamous lung cancer in the CheckMate 017 and 057 studies, respectively (Table 1; refs. 1, 2). An updated survival analysis found a median OS of 9.2 versus 6.0 months for patients with squamous NSCLC treated with nivolumab versus docetaxel and a median OS of 12.2 versus 9.5 months for patients with nonsquamous NSCLC treated with the same agents (3).

On this basis, nivolumab was approved by the FDA for the treatment of advanced NSCLC (both squamous and nonsquamous histologies; ref. 4). An early sign that expression of PD-L1, a PD-1 ligand expressed in a fraction of NSCLCs, might not be a high-performance predictive biomarker was the association of tumor cell (TC) PD-L1 expression with improved response rates and prognosis in nonsquamous histologies but not in patients with squamous cell carcinoma (1, 2).

Pembrolizumab, also a PD-1 inhibitor, was granted accelerated approval by the FDA based on the finding of an objective response rate (ORR) of 19.4% in previously treated NSCLC patients in a large phase I study (KEYNOTE-001). In KEYNOTE-010, the subsequent phase II/III trial of pembrolizumab 2 mg/kg, pembrolizumab 10 mg/kg, or docetaxel in NSCLC patients whose tumors had at least 1% PD-L1 expression (tumor proportion score or TPS $\geq$1%), OS was significantly longer for pembrolizumab at both dose levels than for patients receiving docetaxel (2 mg/kg median OS 10.4 months; 10 mg/kg median OS 12.7 months; docetaxel median OS 8.5 months; ref. 5). In patients with PD-L1 TPS of at least 50%, the advantage of pembrolizumab was even greater at both dose levels (5).

Concurrent work demonstrated the value of inhibiting PD-L1 (6). Clinical responses appear to be correlated with the expression of PD-L1 on both TCs and tumor-infiltrating immune cells (IC). A pooled analysis of patients with multiple tumor types treated on the phase I dose escalation and dose expansion study of atezolizumab, a PD-L1 inhibitor, found that responses were associated with high PD-L1 expression, especially in ICs (7). In the dose expansion portion in patients with NSCLC, an ORR of 23% was observed with increasingly higher ORR among patients with higher PD-L1 expression on TCs and/or ICs (8).

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Table 1. Seminal studies of ICIs in NSCLC

Study name	Phase	Population	Line of treatment	Design	SOC outcome (95% CI)	Intervention outcome (95% CI)	Hazard ratio (95% CI; *P* value)
Later-line metastatic disease							
CheckMate 017	III	Squamous	Second or later	Nivolumab vs. docetaxel	mOS 6.0 months (5.1–7.3)	mOS 9.2 months (7.3–12.6)	0.62 (0.47–0.80)
CheckMate 057	III	Nonsquamous	Second or later	Nivolumab vs. docetaxel	mOS 12.2 months (9.5–15.1)	mOS 9.5 months (8.1–10.7)	0.75 (0.63–0.91)
KEYNOTE-010	II/III	NSCLC PD-L1 TPS ≥1%	Second or later	Pembrolizumab 2 mg/kg or 10 mg/kg vs. docetaxel	mOS 8.5 months (7.5–9.8)	mOS 2 mg/kg 10.4 months (9.4–11.9) mOS 10 mg/kg 12.7 months (10.0–17.3)	2 mg/kg vs. docetaxel: 0.71 (0.58–0.88); $P = 0.0008$ 10 mg/kg vs. docetaxel: 0.61 (0.49–0.75); $P < 0.0001$
POPLAR	II	NSCLC	Second or later	Atezolizumab vs. docetaxel	mOS 9.7 months (8.6–12.0)	mOS 12.6 months (9.7–16.4)	0.73 (0.53–0.99); $P = 0.04$
OAK	III	NSCLC	Second or later	Atezolizumab vs. docetaxel	mOS 9.6 months (8.6–11.2)	mOS 13.8 months (11.8–15.7)	0.73 (0.62–0.87); $P = 0.0003$
First-line metastatic disease							
KEYNOTE-024	III	NSCLC PD-L1 TPS ≥50%	Previously untreated	Pembrolizumab vs. chemotherapy	mOS 14.2 months (9.8–19.0)	mOS 30.0 months (18.3–NR)	0.63 (0.47–0.86); $P = 0.002$
KEYNOTE-042	III	NSCLC PD-L1 TPS ≥1%	Previously untreated	Pembrolizumab vs. chemotherapy	mOS 12.1 months (11.3–13.3)	mOS 16.7 months (13.9–19.7)	0.85 (0.71–0.93); $P = 0.0018$
CheckMate 026	III	NSCLC PD-L1 TPS ≥1%	Previously untreated	Nivolumab vs. chemotherapy	mOS 13.2 months (10.7–17.1)	mOS 14.4 months (11.7–17.4)	1.02 (0.80–1.30)
MYSTIC	III	NSCLC OS reported for PD-L1 TPS ≥25%	Previously untreated	D vs. D + Tr vs. chemotherapy	mOS 12.9 months (10.5–15.0)	mOS 16.3 months D (12.2–20.8) OS 11.9 months D + Tr (9.9–17.7)	D vs. chemotherapy: 0.76 (0.56–1.02) D + Tr vs. chemotherapy: 85 (0.61–1.17)
First-line chemotherapy–checkpoint inhibitor combinations							
KEYNOTE-189	III	Nonsquamous	Previously untreated	C/Pem ± pembrolizumab vs. placebo	12-month OS 49.4% (42.1–56.2)	12-month OS 69.2% (64.1–73.8)	0.49 (0.38–0.64); $P < 0.001$
IMpower 150	III	Nonsquamous; allowed *EGFR/ALK* alterations	Previously untreated	B/C/Pac ± atezolizumab	mOS 14.7 months (13.3–16.9)	mOS 19.2 months (17.0–23.8)	0.78 (0.64–0.96); $P = 0.02$
IMpower 132	III	Nonsquamous	Previously untreated	Pem/P ± atezolizumab	mPFS 5.2 months (4.3–5.6)	mPFS 7.6 months (6.6–8.5)	0.60 (0.49–0.73); $P < 0.0001$
KEYNOTE-407	III	Squamous	Previously untreated	C/T ± pembrolizumab	mOS 11.3 months (9.5–14.8)	mOS 15.9 months (13.2–NR)	0.64 (0.49–0.85); $P < 0.001$
IMpower 131	III	Squamous	Previously untreated	C/Nab ± atezolizumab	mPFS 5.6 months (5.5–5.7)	mPFS 6.3 months (5.7–7.1)	0.715 (0.603–0.848); $P = 0.0001$
Locally advanced setting							
PACIFIC	III	Stage III NSCLC	After definitive chemoRT	D. vs. placebo	24-month OS 55.6% (48.9–61.8)	24-month OS 66.3% (61.7–71.4)	0.68 (99.73% CI, 0.47–0.997); $P = 0.0025$

Abbreviations: B, bevacizumab; C, carboplatin; chemoRT, chemoradiation; CI, confidence interval; D, durvalumab; mOS, median overall survival; mPFS, median progression-free survival; Nab, nab-paclitaxel; NSCLC, non–small cell lung cancer; NR, not reached; P, platinum; Pac, paclitaxel; Pem, pemetrexed; SOC, standard of care; T, taxane; TPS, tumor proportion score; Tr, tremilimumab.

FDA approval of atezolizumab for patients with metastatic NSCLC with disease progression during or after platinum-based chemotherapy was based on the results of the phase II POPLAR trial and phase III OAK trial (9). POPLAR, a phase II open-label trial that randomized patients with previously treated advanced NSCLC to atezolizumab or docetaxel, identified an improved OS in the atezolizumab arm (12.6 vs. 9.7 months), which was associated with PD-L1 expression in both TCs and ICs (10). OAK, a randomized, open-label phase III trial of atezolizumab versus docetaxel in patients with advanced NSCLC after at least one line of platinum-containing chemotherapy, also found that atezolizumab significantly improved OS (13.8 vs. 9.6 months). Moreover, patients with the highest TC and IC PD-L1 expression derived the most benefit (11).

Development of durvalumab, another PD-L1 inhibitor, has confirmed the unreliable nature of PD-L1 expression as an indicator of clinical benefit. In ATLANTIC, a single-arm, open-label phase II trial of durvalumab in patients with previously treated, advanced NSCLC, ORR was associated with tumor PD-L1 expression (12). However, 6.6% of patients whose tumors were negative for PD-L1 experienced an objective response, again suggesting that PD-L1 is an imperfect predictive biomarker. To date, only nivolumab, pembrolizumab, and atezolizumab have been FDA approved for single-agent use in the metastatic setting.

Checkpoint Inhibitors for First-Line Treatment of Metastatic Disease

ICIs were next investigated in the first-line metastatic setting. Again, results were varied in ways that implicated PD-L1 as a limited biomarker. KEYNOTE-024 randomized patients with untreated, advanced NSCLC with PD-L1 TPS of $\geq$50% to pembrolizumab versus investigator's choice of platinum-based chemotherapy (13). In the pembrolizumab group, both median progression-free survival (PFS) and 6-month OS were improved compared with chemotherapy, leading to the FDA approval of pembrolizumab for this population (14). Follow-up analysis has identified an OS benefit for patients in the pembrolizumab group compared with those in the chemotherapy group (median OS 30.0 vs. 14.2 months; ref. 15). Notably, this benefit was observed despite the crossover of 82 of 151 patients in the chemotherapy arm to receive pembrolizumab and was seen in both squamous and nonsquamous histologies.

Expanding this same design to patients with PD-L1 TPS $\geq$1%, KEYNOTE-042 found an OS benefit in all patients, with a median OS of 16.7 versus 12.1 months (16). However, an exploratory analysis of patients with a PD-L1 TPS of $\geq$1%–49% found no meaningful difference in OS in this population, suggesting that single-agent, first-line therapy with pembrolizumab should be limited to patients with a TPS of 50% or greater. However, in patients who are not candidates for chemotherapy based on performance status or other comorbidities, pembrolizumab alone may be a reasonable option.

Somewhat surprisingly, these benefits were not mirrored in CheckMate 026, a phase III study of nivolumab versus platinum-based chemotherapy in patients with untreated advanced NSCLC with a PD-L1 TPS of $\geq$1% (17). In the primary efficacy analysis population of patients with a PD-L1 TPS of $\geq$5%, no significant difference was seen in either PFS or OS. Moreover, exploratory subgroup analyses did not find any significant difference in PFS or OS between the groups in patients with a PD-L1 TPS of $\geq$50%. It is unclear why these results differed from those of KEYNOTE-024. Possibilities include a greater than expected OS in the chemotherapy arm (13.2 months), fewer women in the chemotherapy group, and greater baseline tumor burden in the nivolumab group. Thus, pembrolizumab remains the only FDA-approved ICI available for first-line, single-agent use.

Toxicity Profile of ICIs

Although ICIs are generally well tolerated, they are not without toxicities. The majority are immune-related adverse events (irAE) that result from nonspecific activation of the immune system and induction of autoimmune tissue destruction or alteration, although mechanisms differ based on the organ(s) involved (18). Immune-related toxicities range from the more commonly seen hypothyroidism or skin rash to rarer and more serious manifestations such as colitis, pneumonitis, autoimmune hepatitis, and encephalitis. One study found that 7%–13% of NSCLC patients treated with PD-1 axis inhibitors experienced grade 3 or higher toxicities; the incidence of high-grade irAEs among patients with all tumor types treated with PD-1 and PD-L1 inhibitors is thought to be less than 20% (18, 19). In clinical trials, up to 2% of patients treated with these agents have died from therapy-related toxicities (18). Fortunately, the majority of irAEs can be successfully managed with corticosteroids and other adjunctive medications.

Combining ICIs and Cytotoxic Chemotherapy

Despite the advances described above, only a minority of tumors respond to single-agent immune-checkpoint inhibition (20). Even in KEYNOTE-024, which enrolled nonsquamous patients with a TPS of $\geq$ 50%, the ORR was only 44.8% (13). More recently, combinations of ICIs and chemotherapy, as well as combinations of ICIs, have been investigated with encouraging results.

Combining ICIs with chemotherapy has the potential for synergy through a multitude of mechanisms, including improving antigen presentation to T cells and eliminating immunosuppressive elements of the tumor immune microenvironment (21). The first signal that chemotherapy combined with an ICI might improve outcomes was observed in Cohort G of KEYNOTE-021, a phase II, open-label study that randomized treatment-naïve patients with advanced nonsquamous NSCLC to carboplatin and pemetrexed with or without concomitant pembrolizumab (22). Notably, the primary outcome of ORR was achieved in 55% of patients on the immune combination versus 29% of those on chemotherapy alone, leading to the FDA approval of pembrolizumab in this setting (23).

The results of KEYNOTE-189, a phase III trial that randomized previously untreated patients with metastatic nonsquamous NSCLC to receive carboplatin and pemetrexed with pembrolizumab or placebo, confirmed these findings. The pembrolizumab combination improved both PFS (8.8 vs. 4.9 months) and 12-month OS (69.2% vs. 49.4%) compared with chemotherapy alone (24). This survival advantage was observed among patients with both positive and negative tumor PD-L1 expression (with TPS of <1%, $\geq$1%, 1%–49%, $\geq$50%) and persisted despite the crossover of 41.3% of patients in the intent-to-treat

chemotherapy-only population. Grade 3 or higher adverse events occurred in 67.2% of patients in the pembrolizumab combination group and in 65.8% of patients receiving chemotherapy alone, suggesting little toxicity is added with the use of an ICI.

Two studies have examined the role of atezolizumab combined with chemotherapy for the first-line treatment of metastatic nonsquamous NSCLC. IMpower 150 randomized patients to three groups: atezolizumab, bevacizumab, carboplatin, and paclitaxel (ABCP); ACP; and BCP; the data derived from comparing ABCP and BCP are now available. In patients without *EGFR* and *ALK* alterations, both the median PFS and OS were improved in the atezolizumab-containing group (median PFS 8.3 vs. 6.8 months; median OS 19.2 vs. 14.7 months) compared with the group treated with chemotherapy and bevacizumab (25). Patients with all levels of PD-L1 expression in both tumor and ICs benefited, with benefit increasing with higher expression. However, patients with 0% PD-L1 TC and IC expression had improved survival as well. Interestingly, patients with *EGFR* and *ALK* alterations were also found to benefit from combination therapy with atezolizumab [hazard ratio (HR) 0.59; 95% confidence interval (CI), 0.37–0.94]; as we will discuss, the vast majority of ICI studies in NSCLC have excluded patients with these alterations. A higher incidence of grade $\geq$3 adverse events was observed in the atezolizumab combination group (55.7% vs. 47.7%), primarily consisting of nausea, anorexia, diarrhea, neutropenia, febrile neutropenia, and thrombocytopenia. On the basis of these data, the FDA approved ABCP for the first-line treatment of patients with metastatic nonsquamous NSCLC without *EGFR* or *ALK* alterations (26).

IMpower 132 also examined the role of atezolizumab in first-line chemotherapy combinations for treatment-naïve stage IV nonsquamous NSCLC. PFS was improved in the atezolizumab-containing group (7.6 vs. 5.2 months) and benefit was seen in patients with both positive and negative PD-L1 TC and IC expression (27). However, mature OS data are not yet available.

Based on the above studies, it is clear that both pembrolizumab and atezolizumab in combination with chemotherapy improve OS in the first-line treatment of metastatic nonsquamous NSCLC. However, the latter can only be used with bevacizumab for the time being, reflecting the design of IMpower 150. Additionally, no direct comparison has been made between pembrolizumab alone and in combination with chemotherapy in patients with a PD-L1 TPS of $\geq$50%, making single-agent use of pembrolizumab still appropriate in this setting.

PD-(L)1 inhibitors have also been shown to improve outcomes in treatment-naïve patients with metastatic squamous NSCLC in combination with chemotherapy. In KEYNOTE-407, patients with untreated metastatic squamous NSCLC were randomized to receive 4 cycles of carboplatin and a taxane with or without pembrolizumab (28). Patients in the pembrolizumab-containing group had a significantly improved median OS compared with those receiving chemotherapy alone (15.9 vs. 11.3 months) despite the crossover of 31.7% of the chemotherapy-only group in the intent-to-treat population. Benefit was seen in all PD-L1 TPS groups, leading to FDA approval and establishing this triplet as a new standard of care for this patient population (29). Grade $\geq$3 adverse events were seen in 69.8% of patients receiving pembrolizumab versus 68.2% of those treated with chemotherapy alone, suggesting that the addition of pembrolizumab did not significantly increase toxicity.

Data from the IMpower 131 study suggest that atezolizumab in combination with chemotherapy may also have a role to play in the first-line management of metastatic squamous cell lung cancer (30). Patients who received atezolizumab with carboplatin and nab-paclitaxel had increased median PFS compared with those who received chemotherapy alone (6.3 vs. 5.6 months); this benefit was found at all levels of PD-L1 expression but was most pronounced in those with the highest levels of TC and IC expression. The interim OS analysis did not demonstrate any significant difference in survival, but longer follow-up is pending. Although these data are encouraging, pembrolizumab is the only ICI that has shown an OS benefit to date in combination with chemotherapy for the first-line management of metastatic squamous NSCLC.

PD-L1: An Imperfect Biomarker

PD-L1 IHC was the first FDA-approved companion diagnostic test for ICIs. The IHC assay is done in a standard manner used by other IHC companion diagnostic tests such as ER and HER2 in breast cancer (31) and is read by pathologists, who estimate the percentage of TCs with an intensity of membranous expression (the TPS) and the percentage of ICs with similar expression (the IC proportion score). Currently, four PD-L1 assays are FDA approved in lung cancer. Several studies have compared the sensitivity and reproducibility of these assays for detecting PD-L1 expression in both TCs and ICs (32). Only two of these—a study sponsored by the National Comprehensive Cancer Network and the Blueprint Project—have been prospectively statistically powered. These studies had concordant main conclusions that (i) SP142 shows lower sensitivity than the other FDA-approved assays and a popular laboratory developed assay; and (ii) that pathologists can concordantly read PD-L1 expression on TCs, but do not, even with training, concordantly read PD-L1 expression on ICs (33, 34).

The 22C3 assay is FDA approved as a companion diagnostic for pembrolizumab, although the others are approved as complementary (35). The predictive characteristics of these assays are limited; as we have seen, benefit is often seen in patients whose tumors do not express PD-L1, and many patients whose tumors do express PD-L1 do not derive benefit from PD-(L)1 blockade. This somewhat confusing, and fairly nonspecific test is likely to evolve as the mechanism of action for PD-1 axis therapies is better understood. For the time being, PD-L1 should be used with these caveats as a rough indicative biomarker of response to ICIs and to select patients who are eligible to receive first-line, single-agent pembrolizumab for advanced nonsquamous NSCLC.

Tumor Mutational Burden as a Potential Indicative Biomarker

Recently, tumor mutational burden (TMB) has arisen as another potential indicator of response to immune-checkpoint therapy. TMB refers to the number of somatic mutations found by DNA sequencing of a tumor specimen. The premise of this test is that an increased number of nonsynonymous mutations result in the production of unique tumor neoantigens that can be recognized by the immune system, favoring tumor

recognition and killing by adaptive ICs upon reinvigoration with ICIs (36, 37). This relationship has been demonstrated in retrospective analyses of clinical trials such as CheckMate 026 (38), a subset of patients treated on the POPLAR and OAK trials (39), and retrospective non-trial cohorts (40, 41). To date, these data demonstrate clinical benefit with respect to ORR or PFS, rather than OS. An association between mutational burden and sensitivity to ICIs is also evident in the so-called hypermutated tumors of patients with deleterious alterations in DNA-repair genes such as *MLH1*, *MSH2*, *MSH6*, and *PMS2*, which are characterized by increased $CD8^+$ T-cell infiltrates (42, 43), as well as malignancies with mutations in *BRCA2* (44), *POLD1*, and *POLE* (45).

Combining ICIs

Based on these data, investigators have examined prospectively how TMB might serve as an indicative biomarker for response to ICIs in NSCLC. In CheckMate 227, treatment-naïve patients with stage IV or recurrent NSCLC were randomized to therapies based on PD-L1 TPS; the data comparing nivolumab plus ipilimumab (an inhibitor of cytotoxic T-lymphocyte–associated antigen-4) to chemotherapy alone are now available. In this study, high TMB was defined as $\geq$10 mut/Mb of genome examined (46).

The study demonstrated a PFS benefit of ipilimumab plus nivolumab compared with chemotherapy in patients with tumors with high TMB (and all PD-L1 TPS groups). Median PFS in the high TMB group was 7.2 versus 5.5 months for patients receiving chemotherapy (46). This relationship persisted in patients with high TMB and PD-L1 $\geq$1% and in those with high TMB and PD-L1 <1%; it was also seen in both squamous and nonsquamous histologies, although the benefit appeared greater in patients with nonsquamous NSCLC. No significant PFS improvement was seen in patients with low TMB. An October 2018 Bristol-Meyers Squibb press release revealed that the HR and 95% CIs for patients with both high and low TMB crossed or included 1.0 (47). Thus, it is difficult to make a case for utilizing this combination at this time given the clear OS improvement in chemotherapy–ICI combinations in patients with both squamous and nonsquamous histologies, although more complete data are pending.

It is unclear why TMB does not reliably predict response to PD-1 inhibition with respect to OS, even though it appears to predict for improved PFS. The reasons for this discordance are unknown, but could be related to statistical trial considerations or to the fact that tumors with increased mutations and genomic instability can adapt more quickly to immune pressure, resulting in resistance to immune therapy. Moreover, TMB is neither a sensitive nor specific biomarker, with at least two studies showing area under the ROC curve in the 0.6 range (40, 48). A more concerning issue is the lack of standardization among the many assays that have been used to determine TMB. Although TMB is nonredundant and possibly complementary to PD-L1 by IHC, it uses much more tissue and costs at least 10 times as much per patient assay. Therefore, it may not be a scientifically or financially effective means of predicting response to PD-1 axis therapy. Future prospective randomized studies will be required to assess the clinical value of TMB as a potential biomarker for response to ICIs and to define standards for the assay and metric.

Tumor Inflammation and Outcomes after Checkpoint Inhibition

Measurement of T cells and other immune populations in tumor specimens is also under active development as both a predictive pharmacodynamic biomarker and an endpoint. In multiple tumor types, the presence of prominent T-cell infiltration and IFNγ-related mRNA signatures (indicative of increased adaptive antitumor responses) at baseline have been consistently associated with increased sensitivity to ICIs and improved prognosis (7, 49–52). In patients with advanced NSCLC, detection of increased $CD8^+$ tumor-infiltrating lymphocytes (TIL) by IHC or CD8A mRNA transcripts in baseline tumor specimens is associated with significantly longer PFS after treatment with PD-(L)1 inhibitors; this association was strengthened when combined with PD-L1 at the protein and mRNA level, suggesting that integration of these biomarkers may provide increased predictive value (53). Another study using multiplexed quantitative immunofluorescence to measure TILs in formalin-fixed, paraffin-embedded (FFPE) tumor specimens identified a significant association among baseline $CD3^+$ levels, durable clinical benefit and OS in NSCLC patients treated with ICIs (41).

This study also identified a fraction of T-cell inflamed tumors with limited sensitivity to ICIs, characterized by lower T-cell proliferation (T-cell Ki-67) and cytolytic markers (granzyme B in T cells), suggesting that not all inflamed tumors are equal. Similar findings have been recently reported using a 18-gene inflammation mRNA signature in patients with advanced solid tumors. Although a higher inflammation score was associated with sensitivity to pembrolizumab, a prominent fraction of tumors displaying high scores were resistant to therapy (48).

Other work has utilized blood-based biomarkers to characterize tumor inflammation and response to ICIs. One study examined peripheral blood samples from a cohort of 29 patients with advanced NSCLC treated with PD-1 axis inhibitors and found that 80% of patients who experienced a partial response by RECIST 1.1 criteria had an increase in circulating $PD\text{-}1^+$ $CD8^+$ T cells within 4 weeks of starting therapy, whereas patients whose peripheral $CD8^+$ responses occurred after the 6-week mark did not respond to therapy (54). In a cohort of 100 patients with advanced melanoma and lung cancer being treated with PD-1 inhibitors, patients with objective responses had more baseline peripheral Bim^+ (BH3-only protein, which is downstream of the PD-1–PD-L1 interaction and induces T-cell apoptosis) $CD8^+$ T cells compared with those who did not (55, 56).

Standardization of optimal assays and platforms to reliably measure inflammation as a predictive biomarker for immunotherapy is ongoing, and prospective studies to demonstrate its value are needed.

ICIs in Patients with *EGFR* and *ALK* Alterations

Although patients with tumors harboring sensitizing alterations in *EGFR* and *ALK* have the option of being treated with a variety of targeted agents, PD-1 and PD-L1 inhibitors appear to have limited applicability in this population. Although most clinical trials have excluded these patients, a retrospective analysis found that only 1 of 28 such patients responded to PD-1 or PD-L1

inhibitors, compared with 23.3% of patients with EGFR- and ALK-negative tumors or tumors with unknown EGFR/ALK status (57). A meta-analysis of 5 trials including 3,025 patients with advanced NSCLC treated with a PD-(L)1 inhibitor found that among patients with *EGFR* mutations, OS was not improved compared with docetaxel (58). Recently, a phase II study of first-line pembrolizumab in patients with *EGFR*-mutated, PD-L1–positive (≥1%) advanced NSCLC was terminated early due to lack of efficacy (0 of 11 patients had an objective response), making it clear that ICIs are minimally effective in this setting (59).

IMpower 150, which permitted patients with *EGFR* and *ALK* alterations to enroll, found a benefit of atezolizumab plus chemotherapy in patients with metastatic, nonsquamous NSCLC (25), suggesting that ICI–chemotherapy combinations may have a role in these patients. An ongoing study, KEYNOTE-789, further explores this hypothesis by randomizing patients with *EGFR*mt metastatic NSCLC who have progressed on *EGFR*-specific tyrosine kinase inhibitors (TKI) to receive platinum chemotherapy and pemetrexed with or without pembrolizumab (NCT03515837). Early-phase trials combining TKIs and PD-1 axis inhibitors have largely been disappointing to date. Response rates do not appear to exceed those of single-agent TKIs and the incidence of toxicities, specifically elevated transaminases and interstitial lung disease, exceeds those observed with either agent alone (60–64). Recently, Schoenfeld and colleagues showed that sequential PD-1 axis blockade followed by osimertinib therapy is associated with severe irAEs, especially in patients who begin osimertinib therapy within 3 months of discontinuing PD-(L)1 antagonists (65). Thus, a reliable role for ICIs in NSCLC with *EGFR* and *ALK* alterations has yet to be demonstrated, and these agents may be on the whole more detrimental than beneficial in this population, at least in certain scenarios.

Locally Advanced and Resectable NSCLC

Given the high risk of metastatic spread after definitive treatment of stage III NSCLC, a major goal of ongoing research has been to improve upon the current standard of care to prevent metastatic and ultimately incurable recurrence of locally advanced disease. PACIFIC, a phase III randomized, double-blind trial of durvalumab versus placebo in patients with stage III NSCLC who have completed definitive chemoradiation (66), found that durvalumab improved median PFS by 11.2 months, leading to the FDA approval and adoption of durvalumab after concurrent chemoradiation as a new standard of care (67). Recently published OS data showed a 24-month OS of 66.3% in the durvalumab group and 55.6% in the placebo group (68). Ongoing studies are evaluating the role of PD-1 and PD-L1 blockade after definitive surgical resection and adjuvant chemotherapy for locally advanced NSCLC (NCT02595944 and NCT02504372).

The newest application of ICIs in NSCLC is neoadjuvant use for patients with resectable disease. Retrospective data in NSCLC patients suggest that patients who have a significant pathologic response to neoadjuvant chemotherapy have improved OS (69). Preclinically, murine breast cancer xenografts treated with neoadjuvant anti–PD-1 therapy had improved OS compared with those who received anti–PD-1 therapy adjuvantly (70). The neoadjuvant xenografts also had enhanced numbers of tumor-specific $CD8^+$ cells, suggesting that neoadjuvant immune-checkpoint inhibition may favor T-cell priming to recognize tumor antigens or migration of antigen-specific T cells into the tumor. Theoretically, these T cells could clonally expand and eliminate micrometastases or isolated TCs before they become clinically significant.

A pilot study of neoadjuvant nivolumab in 21 patients with resectable early-stage NSCLC found that nivolumab was well tolerated in this setting (71). Only two partial responses were observed, but on resection, 9 of 20 (45%) patients had a major pathologic response (defined as ≤10% residual tumor present), suggesting that imaging alone may not provide sufficient evidence of treatment response. Multiple phase II and III studies of neoadjuvant immune-checkpoint inhibition in resectable NSCLC are ongoing (NCT02818920, NCT03425643, NCT02259621, NCT03081689, NCT02998528, NCT03158129, NCT03456063, and NCT02927301).

Overcoming Primary and Acquired Resistance to Immune-Checkpoint Inhibition

Although a population of patients with NSCLC clearly derive durable benefit from therapy with ICIs, the sobering fact remains that a large group of patients do not respond to PD-(L)1

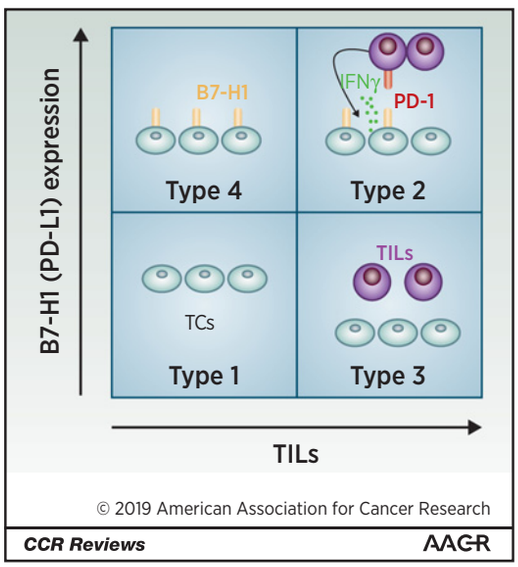

Figure 1.

TIME classification. Four different molecular groups according to B7-H1 (PD-L1) expression (y-axis) and the presence of TILs (x-axis) in tumor biopsies: (1) B7-H1–negative tumors without TILs, considered immunologically ignorant because ICs do not accumulate at the tumor site; (2) PD-L1–positive tumors with TILs, considered a paradigm of adaptive resistance of tumors mediated by the PD-1 pathway; (3) PD-L1–negative tumors with TILs, characterized by tolerance because TILs are present but do not produce IFNγ to induce PD-L1 expression in the tumor microenvironment; and (4) PD-L1–positive tumors without TILs, which result in intrinsic induction of PD-L1 expression in TCs through a variety of oncogenic pathways. B7-H1, B7-homolog 1; PD-1, programmed death-1; TCR, T-cell receptor; TIL, tumor-infiltrating lymphocyte.

inhibition at all (primary resistance). Even in large phase III studies of ICIs combined with chemotherapy, overall response rates are 47%–63% at best (24, 25, 28).

Mechanisms of primary resistance can be understood conceptually by classifying the tumor microenvironment of lung cancer into four subgroups based on the presence or absence of TILs and the presence or absence of PD-L1 expression. This was first done in melanoma (72) and is referred to as the Tumor Immune Microenvironment (TIME) classification (Fig. 1; ref. 73). In many cases, the expression of PD-L1 co-occurs with the presence and activation of TILs (type 2), which characterizes the TIME of tumors in patients most likely to benefit from PD-1 axis inhibitors. In some patients, PD-L1 is constitutively expressed by TCs in the absence of TILs (type 4). In others, a lack of PD-L1 expression may be associated with the reduced presence or absence of detectable TILs (type 1) or the presence of dysfunctional TILs that are not able to mount effective antitumor responses (type 3).

Ideally, these varying immune-evasion mechanisms might be targeted in order to reinvigorate an absent or stunted antitumor immune response. Patients with type 1 and type 4 immune responses have limited TILs in the tumor microenvironment, which can result from defects in tumor antigen presentation or IC trafficking and infiltration. Type 3 responses, which are characterized by defective TIL activation, can result from the absence of tumor-specific T cells, defects in antigen presentation by TCs, or the existence of alternative dominant immune-evasion mechanisms. In these patients, identification and modification of the immune-regulatory pathway that is preventing TIL activation may produce sensitivity to immune-checkpoint inhibition. Ultimately, the goal is to convert all patients' tumor microenvironments to that seen in type 2, in which T-cell tumor infiltration and activation occur, IFNγ is released, and PD-L1 is expressed, rendering tumors sensitive to PD-1 axis inhibitors. Lung-MAP, an umbrella trial of multiple therapies for patients with squamous cell lung cancer based on their tumor mutational profiles, has recently been redesigned to focus on patients with primary resistance to ICIs (74).

Resistance to ICIs can also develop after a patient has experienced a documented response (acquired resistance). Although ICIs have been shown to give rise to durable responses in some patients with NSCLC, the median duration of response is between 1 and 2 years, with the majority of patients developing resistance to the therapies after an initial response (3, 11, 75).

Due to the limited amount of time that ICIs have been used in the clinic and the shortage of patient samples to analyze mechanisms of resistance, much remains unknown about the molecular mechanisms driving acquired resistance to these drugs. Loss-of-function mutations in and homozygous deletion of β2-microglobulin (*B2M*) that result in defective HLA I antigen presentation have been reported in tumors at resistance to ICIs and other immunotherapies in lung cancer, melanoma, and colon cancer (75–79). Reduced HLA I antigen presentation without genetic loss is also possible, which suggests that mechanisms other than genomic changes can lead to resistance (75).

Defects in tumor HLA I antigen presentation can occur in many other ways. Copy-number loss of genomic regions encoding antigenic mutations or specific HLA alleles has been described as a mechanism of acquired resistance to immunotherapies in multiple tumor types, including lung cancer (80, 81).

Loss-of-function mutations in components of IFN signaling pathways such as *JAK1/2* have been identified in cases of acquired resistance to ICIs in melanoma (76). A number of TC-intrinsic signaling pathways implicated in the setting of primary resistance may also be relevant to acquired resistance, including upregulation of PD-L1 (82, 83), loss of *PTEN* (84, 85), loss-of-function mutations of *STK11/LKB1* (86, 87), activation of *c-Myc* (88), activation of the WNT/β-catenin pathway (89), and alterations in the chromatin regulator PBAF (90, 91).

T-cell–dependent mechanisms have also been associated with ICI resistance, including insufficient T-cell activation (92), upregulation of additional immune checkpoints (e.g., TIM-3 and LAG-3; refs. 75, 93, 94), and exclusion of T cells from the tumor microenvironment due to lack of appropriate chemokines (95, 96). Future studies to determine the role and significance of these mechanisms of resistance will guide the development of approaches to overcome them or even preempt their emergence.

Novel Targets and Combinations

As we have seen, a detailed picture is emerging of the immune components and immune-inhibitory mechanisms responsible for an effective or ineffective antitumor immune response. Given our growing understanding of the TIME of NSCLC, efforts to exploit this knowledge using novel immunologic targets are under way. Along these lines, clinical trials utilizing agents targeting VISTA/PD-1H (NCT02671955), B7-H4 (NCT03514121), B7-H3 (NCT02628535, NCT02475213, and NCT03406949), LAG-3 (NCT01968109 and NCT02460224), and IL8 (NCT02536469), often in combination with PD-1 axis inhibitors, are ongoing (Fig. 2). In addition to vaccine approaches targeting tumor antigens such as NY-ESO1 and mesothelin or cell therapies, several early-phase studies are exploring the use of a personalized cancer vaccine formulated using individual patient's tumor neoantigens, to treat a variety of advanced solid tumors (e.g., NCT03639714, NCT03480152, and NCT03289962).

Additionally, radiotherapy is being used in combination with ipilimumab to induce an inflammatory TIME with promising results suggestive of a systemic antitumor immune response in patients previously refractory to ipilimumab (97). Other strategies aimed at inducing an inflammatory TIME include T-cell–bispecific antibodies (NCT02324257) and combinations of ICIs and targeted therapies such as inhibitors of poly-ADP ribose polymerase (PARP) and histone deacetylase (HDAC) to modulate TC antigenicity (NCT03377023 and NCT02638090).

Conclusions

The development of PD-1 axis inhibitors in NSCLC, both alone and in combination with chemotherapy, has ushered in a new era in the management of this challenging set of cancers. A growing number of patients are deriving meaningful, durable benefit with a reasonable toxicity profile. Biomarkers such as PD-L1, TMB, or tumor inflammation, though fraught in many ways, offer insight into which patients may benefit most and could be used to optimize the development of new agents and combinations. The next step is to better identify patients at risk

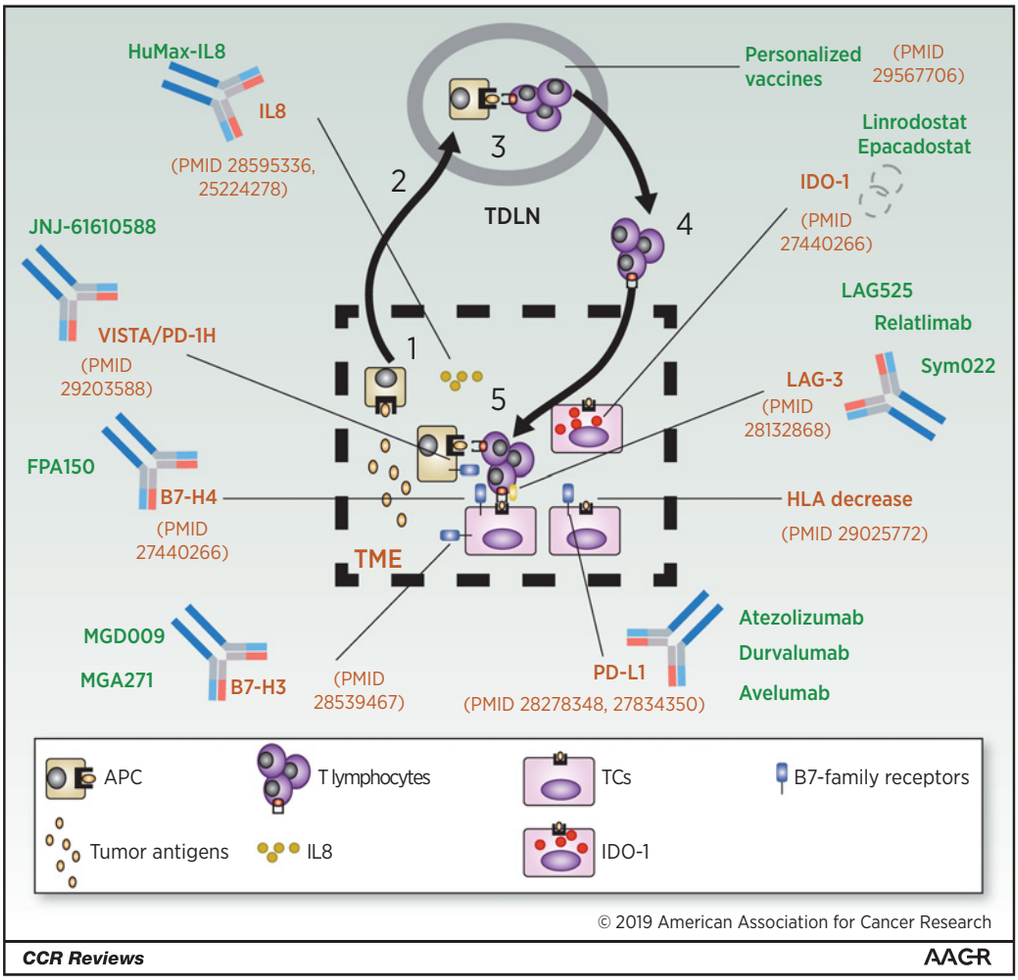

Figure 2.
Immune-escape mechanisms in NSCLC and new immune target opportunities. Different immune-escape mechanisms developed by lung tumors (brown) and available immunotherapies targeting these mechanisms (green) to stimulate an antitumor immune response. (1) Antigen uptake and processing by antigen-presenting cells (APC); (2) migration of APCs to lymphoid organs; (3) antigen presentation, activation, and costimulatory and coinhibitory regulation of naïve T cells to become effector T cells in lymphoid organs; (4) exit of effector T cells into peripheral blood and trafficking to tumor tissues; and (5) tumor antigen recognition and tumor lysis. Targets include IL8, VISTA/PD-1H, B7-H4, B7-H3, IDO-1, LAG-3, HLA class I, and tumor-specific neoantigens. B7-H3, B7-homolog 3; B7-H4, B7-homolog 4; HLA, human leukocyte antigen; IDO-1, indoleamine 2,3-dioxygenase-1; IL8, interleukin-8; LAG-3, lymphocyte-activation gene 3; PD-1H, programmed death-1 homolog; TDLN, tumor-draining lymph node; TME, tumor micorenvironment; VISTA, V-domain Ig suppressor of T-cell activation.

of primary or acquired resistance and use a growing body of translational science to develop combination therapies, making the promise of immune-checkpoint inhibition available to all patients with NSCLC.

Disclosure of Potential Conflicts of Interest

D.B. Doroshow is a consultant/advisory board member for Boehringer Ingelheim and Ipsen. K. Politi reports receiving commercial research grants from AstraZeneca, Roche, Symphogen, and Kolltan, is coinventor on a patent licensed to Molecular MD for EGFR T790M mutation testing (through Memorial Sloan Kettering Cancer Center), and is a consultant/advisory board member for AstraZeneca, Merck, Tocagen, Novartis, Maverick Therapeutics, and Dynamo Therapeutics. D.L. Rimm reports receiving commercial research grants from AstraZeneca and Navigate Biopharma, and is a consultant/advisory board member for Bristol-Myers Squibb, Merck, AstraZeneca, Agilent, and Ventana. L. Chen is scientific founder of, reports receiving commercial research grants from, and holds ownership interest (including patents) in NextCure Inc. and TAYU Biotech, and is a consultant/advisory board member for Pfizer, Vcanbio, GenomiCare, Zai Labs, and Junshi Pharmaceuticals. I. Melero reports receiving commercial research grants from Alligator, Roche, Bristol-Myers Squibb, and Bioncotech, speakers bureau honoraria from MSD, holds ownership interest (including patents) in TUSK, and is a consultant/advisory board member for Bayer, Bristol-Myers Squibb, Roche, Alligator, MedImmune, Numab, Molecular Partners, Bioncotech, and Pieris. K.A. Schalper reports receiving commercial research grants from Surface Oncology, Pierre Fabre, Moderna, Vasculox/Tioma, Bristol-Myers Squibb, Merck, Tesaro, and Navigate Biopharma, speakers bureau honoraria from Takeda and Merck, and is a consultant/advisory board member for Moderna, Celgene, Shattuck Labs, and Pierre Fabre. R.S. Herbst reports receiving commercial research grants from AstraZeneca, Eli Lilly and Company, and Merck and Company, and is a consultant/advisory board member for AbbVie Pharmaceuticals, AstraZeneca, Biodesix, Bristol-Myers Squibb,

Eli Lilly and Company, EMD Serono, Genentech/Roche, Heat Biologics, Infinity Pharmaceuticals, Loxo Oncology, Merck and Company, Nektar, Neon Therapeutics, NextCure, Novartis, Pfizer, Sanofi, Seattle Genetics, Shire PLC, Spectrum Pharmaceuticals, Symphogen, Tesaro, ARMO Biosciences, Genmab, and Tocagen. He is a board member (nonexecutive/independent) for Junshi Pharmaceuticals. No potential conflicts of interest were disclosed by the other authors.

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