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Immunotherapy in Lung Cancer

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Abstract

Lung cancer has not traditionally been viewed as an immune-responsive tumor. However, it is becoming evident that tumor-induced immune suppression is vital to malignant progression. Immunotherapies act by enhancing the patient's innate immune response and hold promise for inducing long-term responses in select patients with non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). Immune checkpoint inhibitors, in particular, inhibitors to cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and programmed death 1 (PD-1) and programmed death receptor ligand 1 (PD-L1) have shown promise in early studies and are currently in clinical trials in both small cell lung cancer and non-small cell lung cancer patients. Two large randomized phase III trials recently demonstrated superior overall survival (OS) in patients treated with anti-PD-1 therapy compared to chemotherapy in the second-line setting.

Keywords

Immunotherapy; PD-1; PD-L1; CTLA-4

1 Cancer and the Biology of Immune Evasion

The immune system provides the primary defense against the development and growth of cancer. Through immunosurveillance, the immune system is able to recognize and eradicate incipient tumor cells [61]. The ability to escape the immune response, therefore, is vital to cancer survival and malignant progression [25]. This evasion may occur through either tumor-directed processes, typically involving alteration in the tumor cells themselves or the tumor microenvironment, or immune system-directed processes in which the tumor induces innate regulatory mechanisms to suppress the immune response [17].

Immunosurveillance involves every aspect of both the innate and adaptive immune system [15]. The innate immune system initiates antitumor immunity when NK cells recognize tumor-specific antigens, leading to destruction of the malignant-transformed cells [13]. Lysed tumor cell fragments are adsorbed and processed by macrophages and dendritic cells. Activation of macrophages and dendritic cells leads to both expression of inflammatory cytokines and presentation of tumor-specific ligands to T and B cells, thereby instigating

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the adaptive immune response [43]. The adaptive immune response involves the generation and expansion of tumor-specific T cells and antibodies [16, 17, 41]. Ideally, these processes culminate in elimination of cancer cells and generation of long-term immune memory [16]. However, it is also possible that a state known as cancer equilibrium may occur, in which the immune system maintains the tumor in a state of functional dormancy [16, 43]. Under this state, tumor cells, exposed to persistent immune pressure, may undergo genetic and epigenetic changes that ultimately can result in the selection of less immunogenic phenotypes [41, 43, 63], thereby facilitating the possibility of immune escape [16].

Evasion of the antitumor immune response occurs both at the level of the tumor cell and the tumor microenvironment. Lung cancer cells may be protected from immune recognition by downregulating proteins involved in antigen presentation, such as the immunoproteasome subunits large multifunctional peptidases 2 and 7 (LMP2 and LMP7), antigen peptide transporters 1 and 2 (TAP1 and TAP2), and the major histocompatibility (MHC) molecules [7]. Additionally, the oncogenic process may lead to multiple genetic and epigenetic alterations, rendering potential lung cancer antigens unstable and allowing for passive immune escape [14]. Such immune escape mechanisms are thought to be particularly important in smoking- and pollution-associated lung cancers, which harbor a high density of somatic mutations and epigenetic dysregulation [23, 63]. The expression of immune inhibitory molecules is another mechanism of immune evasion that has therapeutic importance in lung cancer. Regulatory T cells, which are present at increased numbers in patients with NSCLC, can suppress T cell activation through the production of TGF-B and interleukin-10 [37, 77, 80], thereby inducing immune tolerance. Membrane-bound inhibitory ligands, also known as checkpoint ligands, have amplified expression in lung cancer and include programmed death receptor ligand 1 (PD-L1), PD-L2, B7-H3, and B7-H4 [7, 46]. PD-L1, which is the most studied checkpoint ligand to date, is thought to be expressed in approximately half of NSCLCs, with equal proportion in squamous and nonsquamous histologies [44]. Tumor-infiltrating CD8+ and CD4+ lymphocytes have been identified in resected NSCLC specimens at rate ranging from 25 to 83 % and are thought to have a favorable prognostic significance in resected early-stage disease [28, 34, 49, 58].

Disruption of tumor-induced immune suppression has been a goal of various immunotherapies under development. Tumor-specific antigens that theoretically should enable the immune system to distinguish between malignant and normal cells have been the focus of therapeutic vaccines, with limited success to date. More recently, immune checkpoint inhibitors have shown promising activity in patients with advanced small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). These agents were developed with the goal of overcoming tumor-induced immune suppression and generating potentially durable antitumor immune responses.

2 Immune Checkpoint Inhibitors

Immune checkpoint inhibitors liberate previously repressed antitumor immune responses by modulating the interaction of T cells with either antigen-presenting cells (APCs) or tumor cells. Because the released immune response is thought to encompass immune memory as well, some patients experience apparently durable remissions without evidence of tumor

resistance or relapse. Agents targeting the cytotoxic T-lymphocyte antigen 4 (CTLA-4) and the programmed cell death receptor-1 (PD-1) and its ligand, PD-L1, are currently being evaluated in patients with advanced stage lung cancer.

2.1 Therapies Targeting CTLA-4

CTLA-4 inhibitors were the earliest checkpoint inhibitors to reach clinical development. Activation of cytotoxic T cells entails binding of the T cell receptor with an MHC molecule as well as co-stimulatory signals mediated through CD28 and B7 [41]. The CTLA-4 protein is expressed on the T cell surface and functions as a negative regulator of T cell activation by competing with CD28. Antibodies to CTLA-4 inhibit this crucial negative regulator of T cell activation with the goal of releasing suppressed antitumor immune responses [59, 76]. The resultant immune activation also causes a unique toxicity profile of immune-related adverse events including pneumonitis, colitis, dermatitis, hepatitis, endocrinopathies, and neuropathy.

Ipilimumab is a fully humanized monoclonal antibody directed at CTLA-4 and functions to prevent receptor binding to cognate ligands. It was approved for use in metastatic melanoma after showing significant improvement in overall survival compared to chemotherapy in pretreated patients with metastatic disease [30]. Ipilimumab has subsequently been evaluated at various doses and combinations in lung cancer. A phase II trial of paclitaxel (175 mg/m2) and carboplatin (AUC = 6) with ipilimumab (10 mg/kg) as either phased (two doses of placebo plus chemotherapy followed by four doses of ipilimumab plus chemotherapy) or concurrent (four doses of ipilimumab plus chemotherapy followed by two doses of placebo plus chemotherapy) administration, or placebo, in a treatment-naïve patients with advanced NSCLC resulted in immune-related progression-free survival (irPFS) of 5.7, 5.5, and 4.6 months, and median overall survival (OS) of 12.2, 9.7, and 8.3 months, respectively [42]. This resulted in a statistically significant improvement in irPFS with the phased dosing of ipilimumab as compared to placebo, but not the concurrent dosing schedule, and improvement in OS did not reach statistical significance. Under the phased dosing schedule, patients received two doses of placebo plus paclitaxel and carboplatin, followed by four doses of ipilimumab plus paclitaxel and carboplatin. An unplanned subset analysis of histologic subgroups revealed that both progression-free survival (PFS) and OS were improved in the phased ipilimumab group for patients with squamous histology (HR for progression 0.40 [95 % CI, 0.18–0.87], HR for death 0.48 [95 % CI, 0.22–1.03]) that was not seen in patients with nonsquamous cell histology. Grade 3 and 4 immune-related adverse events (irAEs) including colitis, hepatitis, and hypophysitis occurred at rates of 6, 20, and 15 % in the placebo, concurrent ipilimumab, and phased ipilimumab arms, respectively. A similar phase II trial of phased and concurrent ipilimumab (10 mg/kg) in combination with paclitaxel (175 mg/m^2) and carboplatin (AUC = 6) versus chemotherapy alone was performed in treatment-naïve patients with extensive stage SCLC [51]. Treatments were administered every 3 weeks for a maximum of 18 weeks, and followed by either maintenance ipilimumab or placebo every 12 weeks. This trial also found a statistically significant improvement in irPFS with phased ipilimumab (6.4 months) but not concurrent ipilimumab (5.7 months) as compared to placebo (5.3 months). Median OS was 12.9, 9.1, and 9.9 months with phased ipilimumab, concurrent ipilimumab, and chemotherapy alone,

respectively. Grade 3 and 4 irAEs including rash, colitis, and hepatitis occurred at rates of 9, 21, and 17 % in the placebo, concurrent ipilimumab, and phased ipilimumab arms, respectively. Further study of ipilimumab in lung cancer patients has moved forward with a phase III trial of ipilimumab (10 mg/kg) in combination with carboplatin (AUC = 6) and paclitaxel (175 mg/m2) versus carboplatin and paclitaxel alone in patients with advanced squamous NSCLC (NCT01285609). The combination of ipilimumab with carboplatin and etoposide as a first-line treatment in patients with extensive stage SCLC has completed enrollment and was recently reported as a negative trial (NCT01331525).

Tremelimumab is a fully humanized IgG2 monoclonal antibody to CTLA-4. In contrast to ipilimumab, a large phase III trial in treatment-naïve patients with advanced melanoma did not demonstrate improved PFS, OS, or objective response rate (ORR) compared to cytotoxic chemotherapy although some durable responses were observed and tremelimumab was given as maintenance rather than induction therapy [52]. Single agent tremelimumab in NSCLC has yielded similar results to date. In a phase II trial of 87 patients with advanced NSCLC, tremelimumab was administered as a maintenance therapy following 4 cycles of platinum-based chemotherapy [79]. There was no improvement in PFS in this study (20.9 vs. 14.3 % progression free at 3 months). Approximately 20 % of patients on the tremelimumab arm experienced a grade 3/4 adverse event the most common being colitis (9.1 %). Studies with tremelimumab in combination with anti-PD-L1 therapy and gefitinib in patients with NSCLC are ongoing (NCT02000947; NCT02040064).

2.2 Therapies Targeting PD-1

The PD-1 receptor and its two ligands, PD-L1 (B7-H1) and PD-L2 (B7-DC), negatively regulate T cell activation [38]. The PD-1 receptor is a transmembrane protein that can be expressed on T cells, B cells, natural killer T cells, activated monocytes, and dendritic cells. PD-L1 is expressed by monocytes and lung tissue, as well as vascular endothelium, mesenchymal stem cells, keratinocytes, and activated T cells [38]. PD-L1 is also expressed in approximately half of NSCLCs (both adenocarcinoma and squamous cell histologies) and may be associated with a poor prognosis [44]. Binding of the PD-1 receptor by its ligands leads to inhibition of T cell receptor signaling, downregulation of the PI3 K pathway, and decreased induction of cytokines such as interferon-γ (IFN-γ) [38]. Therapies directed against PD-1 block the interaction of PD-1 with its ligands, thereby activating dormant T cell-mediated immune responses. PD-L1 is frequently found in combination with high levels of tumor-infiltrating lymphocytes, indicating that exhaustion of the antitumor T cell response may aid lung cancer progression and immune evasion [36]. However, this coupling of PD-L1 expression with tumor-infiltrating lymphocytes may help confine therapy-induced T cell activation to the tumor microenvironment, thereby limiting systemic immune-related toxicity [7]. Anti-PD-1 and anti-PD-L1 agents do not induce antibody-dependent cell-mediated cytotoxicity (ADCC), an important consideration as ADCC could potentially deplete activated T cells and tumor-infiltrating immune cells [67]. Antibodies engineered against both PD-1 and PD-L1 are currently in development for use in lung cancer.

Nivolumab (BMS936558) is a human IgG4 monoclonal antibody to PD-1 and is the agent that is furthest in development in its class for NSCLC. Its utility in lung cancer patients was first explored in a large, phase I trial that included multiple expansion cohorts of patients including NSCLC, melanoma, and renal cell carcinoma (RCC) [69]. In this trial, 129 heavily pretreated patients with NSCLC received nivolumab (1, 3, or 10 mg/kg IV every 2 weeks). The ORR for patients with NSCLC across dosing levels was 17.1 %, with no significant difference between patients with squamous (16.7 %; 9 of 54) and nonsquamous histology (17.6 %; 13 of 74). Additionally, 5 % of patients had unconventional immune-pattern responses, and 10 % of patients had stable disease lasting at least 24 weeks. Median OS across doses was 9.9 months, and at the 3 mg/kg dose (which was chosen for use in subsequent trials) the 1-, 2-, and 3- year OS rates were 56, 42, and 27 %, respectively [20, 21]. Drug-related adverse events were seen in 53 % of patients, 6 % of which were grade 3/4 including gastrointestinal, pulmonary (pneumonitis), hepatitis, and infusion reactions. Durable responses were common with a median duration of response of 17 months (range 1.4–36.8 months). Eighteen responders discontinued nivolumab for reasons other than progressive disease, and 9 of these had responses for more than 9 months following therapy cessation [20, 21]. Subset analyses did not reveal any predictive value for EGFR or KRAS mutations as compared with wild-type (Brahmer JR [6]. However, there was predictive value for intratumoral PD-L1 expression (defined as 5 % expression threshold by immunohistochemistry). Of the 25 patients with known PD-L1-positive tumors, 36 % had an objective response versus no response among 17 patients with known PD-L1-negative tumors ($p = 0.006$) [69].

CheckMate 063 was a phase II single-arm trial of nivolumab (3 mg/kg) in patients with advanced, refractory squamous NSCLC[56]. Of the 117 patients enrolled, 17 (14.5, 95 % CI 8.7–22.2 %) had an objective response, and 77 % of those responses were ongoing at time of analysis. An additional 30 patients (26 %) had stable disease, with a median duration of 6.0 months (95 % CI 4.7–10.9 months). The most common grade 3/4 adverse events were fatigue (4 %), pneumonitis (3 %), and diarrhea (3 %). CheckMate-017 was a phase III open-label trial that enrolled 272 previously treated patients with advanced or metastatic squamous cell NSCLC [5]. Patients were randomized to receive either nivolumab (3 mg/kg every 2 weeks) or docetaxel (75 mg/m^2 every 3 weeks). The trial was stopped early when preliminary analyses indicated an overall survival advantage of 3.2 months favoring nivolumab over docetaxel (median OS 9.2 months versus 6.0 months for nivolumab and docetaxel, respectively; hazard ratio 0.59; $p < 0.001$). The median PFS was 3.5 months with nivolumab versus 2.8 months with docetaxel (HR for death or disease progression 0.62; $p < 0.001$), and the overall survival rate at one year was 42 % with nivolumab versus 24 % with docetaxel. At the time of study reporting, the median duration of response in the nivolumab arm had not been reached (range 2.9–20.5 + months), but median duration of response was 8.4 months in the docetaxel arm. Grade 3 and 4 adverse events occurred in 7 % of patients receiving nivolumab, compared to 55 % of patients treated with docetaxel. PD-L1 expression was evaluated in 83 % of patients, but no prognostic or predictive association was found between PD-L1 expression and any efficacy endpoint. These findings led to FDA approval for nivolumab use in patients with refractory or recurrent advanced squamous NSCLC in March 2015. CheckMate-057, a phase III study of nivolumab (3 mg/kg every

2 weeks) versus docetaxel (75 mg/m^2 every 3 weeks) in previously treated patients with advanced or metastatic nonsquamous NSCLC, was also halted early when it was reported to meet its endpoint of improved overall survival [48]. As compared to docetaxel, nivolumab demonstrated superior OS (HR = 0.73, p = 0.00155) and ORR (19.2 vs. 12.4 %; p = 0.0235). The median response duration was 17.1 months (range: 8.4 months—not estimable) in the nivolumab arm, compared to 5.6 months (range: 4.4–7.0 months) in the docetaxel arm. Rates of grade 3–5 toxicity were substantially less in the nivolumab arm compared to docetaxel (10.5 vs. 53.7 %). Interestingly in this trial the median PFS with nivolumab (2.3 months) was inferior compared to chemotherapy (4.2 months), although the difference was not statistically significant (HR 0.92, 95 % CI: 0.77–1.11; p = 0.393). Positive expression of PD-L1 was not a prerequisite to study entry, but subset analysis was performed and higher levels of PD-L1 expression appeared to correlate with improved benefit. CheckMate 026 compared first platinum-based chemotherapy to nivolumab in patients with advanced stage NSCLC with *EGFR* and *ALK* wild-type status and showed tumors that are positive for expression of PD-L1 (NCT02041533).

Nivolumab is also being evaluated in combination with chemotherapy, as well as targeted agents such as erlotinib and bevacizumab. CheckMate-012 is a multi-arm phase 1b trial of nivolumab in combination with multiple agents including ipilimumab and several possible combinations of platinum-based doublet chemotherapy [2]. Patients were assigned to a chemotherapy regimen by histology: Squamous histology got nivolumab (10 mg/kg) plus gemcitabine (1250 mg/m^2) and cisplatin (75 mg/m^2); nonsquamous patients got nivolumab (10 mg/kg) plus pemetrexed (500 mg/m^2) and cisplatin (75 mg/m^2); and patients with any histology got either nivolumab 10 mg/kg or 5 mg/kg plus paclitaxel (200 mg/m^2) and carboplatin (AUC = 6). Early results reported at the 2014 Symposium in Thoracic Oncology demonstrated an ORR of 33, 47, and 47 % and 18-month OS rates of 33, 60, and 40 % for nivolumab 10 mg/kg when combined with gemcitabine/cisplatin, pemetrexed/cisplatin, and paclitaxel/carboplatin, respectively. Preliminary results from a separate study of 21 patients with *EGFR*-mutant NSCLC who received nivolumab plus erlotinib were reported at ASCO 2014 [55]. Of the twenty patients with acquired resistance to erlotinib, 3 patients experienced a PR (15 %) and 9 patients had stable disease (45 %). Grade 3/4 adverse events occurred in 4 patients including 3 with elevations in liver function tests.

Pembrolizumab (MK3475) is another humanized IgG4 anti-PD1 antibody that has shown promise for use in NSCLC patients. KEYNOTE-001 was a large phase I study of pembrolizumab at varying doses in 495 patients with advanced NSCLC [19]. This study also evaluated PD-L1 tumor expression as part of its eligibility criteria; PD-L1 expression levels were assessed with the anti-PD-L1 antibody clone 22C3 and a prototype immunohistochemistry assay developed by Merck. A separate validation group of patients was selected to assess the efficacy of the prototype assay. For this group, PD-L1 expression cutoffs were defined as a proportion score of ≥50 % (strong), 1–49 % (weak), or <1 %. Of the 1143 patients initially screened for the study, 824 had evaluable samples and 23.2, 37.6, and 39.2 % had proportion scores of ≥50 %, 1–49 %, and <1 % by the prototype assay. The ORR was 19.5 %, with similar response rates among previously treated (18 %) and untreated (24.8 %) patients. An additional 21.8 % of patients exhibited stable disease. Responses were more frequent among current or former smokers as compared to never smokers, with ORR

of 22.5 % vs 10.3 %, respectively. The median duration of response was 12.5 months (range 1.0–23.3 months). Median OS was 12.0 months, with better survival in treatment-naïve as compared to previously treated patients (median OS 16.2 months (95 % CI, 16.2 to not reached) and 9.3 months (95 % CI, 8.4–12.4), respectively). There was a positive relationship between PD-L1 expression and survival, as the median PFS (6.3 months) and OS (not reached) were better among patients with a PD-L1 proportion score of at least 50 % than patients with scores of 1–49 % or <1 %. However, duration of response was similar across all proportion scores: 12.5 months (range, 2.1–23.3) for a proportion score of at least 50 %, 7.2 months (range, 1.4–8.3) for a proportion score of 1–49 %, and not reached (range, 1.0–10.8) for a proportion score of less than 1 %. Grade 3/4 treatment-related toxicities were observed in only 9.5 % of patients and included pneumonitis, fatigue, asthenia, and anorexia. There were no significant differences in efficacy or adverse events in patients receiving doses of 10 mg/kg every 2 weeks compared to every 3 weeks; data regarding the lower 2 mg/kg dose were lacking at time of study publication. Although large trials of PD-1 agents generally exclude patients with active CNS disease, early data from a phase II study of NSCLC patients with untreated or progressive brain metastases (size range 5–20 mm) suggest that pembrolizumab has activity against CNS disease, with partial responses seen in 4 of 9 evaluable patients [22].

KEYNOTE-021 is a multi-arm phase II study evaluating the safety, tolerability, and efficacy of pembrolizumab combined with platinum doublet chemotherapy in patients with advanced NSCLC in the first-line setting [45]. Early results demonstrated promising response rates, particularly with the combination of pembrolizumab and carboplatin (AUC = 5) plus pemetrexed (500 mg/m2). Although numbers were small (n = 12 in each arm), patients who received this triplet combination demonstrated an ORR of 67 % and 50 % as well as a disease control rate of 100 and 92 % with this platinum doublet and pembrolizumab at doses of 10 and 2 mg/kg, respectively. As expected, the grade 3/4 toxicity rate of 38 % with this combination was higher than in studies of pembrolizumab monotherapy.

Several studies are ongoing or planned for pembrolizumab, including a single-arm monotherapy trial (NCT01295827), and a phase III trial comparing to docetaxel to pembrolizumab in previously treated patients (NCT01905657). Both of these trials require a biopsy prior to entry on study and are only enrolling patients with tumors that are positive for expression of PD-L1. A phase I/II trial in unselected patients evaluated pembrolizumab in combination with chemotherapy, bevacizumab, tyrosine kinase inhibitors, or ipilimumab (NCT02039674). A first-line trial comparing pembrolizumab- to platinum-based chemotherapy in patients with newly diagnosed NSCLC is also ongoing (NCT02220894).

2.3 Therapies Targeting PD-L1

Several agents that target PD-L1, the ligand for PD-1, are also in development. These agents block the interaction of PD-L1 expressed on tumor cells and tumor-infiltrating immune cells with PD-1 and B7.1 expressed on T cells. The effects of these agents are predicted to be similar to anti-PD-1. It is theorized that this variation in mechanism may lead to different

antitumor and toxicity profiles as compared to the anti-PD-1 agents. At this point, it is not clear which approach is superior.

BMS-936559 was the first PD-L1 antibody to be assessed in NSCLC patients. An ORR of 10 % was observed in 49 evaluable NSCLC patients enrolled in a phase I trial evaluating multiple different dose levels [9]. Clinical development of this agent has been suspended at this time. MEDI-4736 is an anti-PD-L1 antibody undergoing evaluation in a phase I study that includes a subgroup of NSCLC patients, in addition to other solid tumor malignancies. An early report described confirmed partial responses in 3 of 13 heavily pretreated NSCLC patients, with toxicities appearing similar to other anti-PD-L1 agents [8].

Atezolizumab (MPDL3280A) is a human IgG1 monoclonal antibody to PD-L1 that has shown the most promise in the class of agents in NSCLC to date. A phase I study conducted in advanced solid tumors found activity in NSCLC, melanoma, RCC, gastric cancer, and head and neck squamous cell carcinoma. Of the 85 patients with NSCLC included in the study, the ORR was 23 % per RECIST 1.1 criteria, with a higher ORR (83 %) in tumors that were IHC3 positive (defined as staining of 10 % of tumor for PD-L1 expression) [62]. Similar to anti-PD-1 agents, a higher ORR was seen in current/former smokers (26 %; n = 43) as compared to never smokers (10 %; n = 10). Most AEs were of low grade with only 11 % being grade 3/4, and no pneumonitis was observed. On the basis of these early results, the FDA granted atezolizumab Breakthrough Therapy Designation for NSCLC in February 2015. An interim analysis of FIR, a single-arm study of atezolizumab in patients with stage IIIB/IV NSCLC and high PD-L1 expression in either tumor cells or tumor-infiltrating immune cells, reported an ORR of 29 %, and 24 week PFS rate of 45 % [64]. Early results from POPLAR, a phase II study of atezolizumab (1200 mg IV every three weeks) versus docetaxel (75 mg/m2 every three weeks) in previously treated patients with NSCLC, were reported at ASCO 2015. Interim results indicated a nonstatistically significant improvement in median OS with atezolizumab as compared to docetaxel (11.4 vs. 9.5 months; HR 0.77, p = 0.11) in all comers, with greatest benefit seen in patients with high expression of PD-L1 in either tumor cells or tumor-infiltrating immune cells. Rates of grade 3–5 toxicity were lower in the atezolizumab group as compared to docetaxel (43 vs. 56 %, respectively), and immune-mediated adverse events (any grade) included elevated AST (4 %), elevated ALT (4 %), pneumonitis (2 %), colitis (1 %), and hepatitis (1 %) [65]. Ongoing clinical trials include a single agent study in patients with PD-L1-positive tumors (NCT02031458) comparing MDPL3280A to chemotherapy (NCT02008227) and in combination with targeted therapy and bevacizumab in NSCLC patients (NCT02013219).

2.4 Combination Therapies

The promising outcomes and favorable toxicity profile of anti-PD-1/PD-L1 therapy have led to multiple ongoing studies combining these agents with CTLA-4-directed agents, targeted therapies, and chemotherapy. Early results of combination studies available at time of this publication are described below.

Anti-PD-1/PD-L1 and anti-CTLA-4 antibodies activate different aspects of the immune response, and it is thought that they may complement each other therapeutically. Anti-PD-1/PD-L1 therapies target the antigen-presenting cell–T cell interaction, whereas anti-CTLA-4

therapies act at the effector T cell–tumor cell interface [35]. A phase III trial comparing combined ipilimumab and nivolumab therapy to either nivolumab alone or ipilimumab alone in treatment-naïve patients with advanced melanoma found improved median PFS with the combination as compared to ipilimumab alone (11.5 vs. 2.9 months, HR 0.42, $p < 0.001$). Although patients with PD-L1-positive tumors showed improved PFS with the combination versus ipilimumab, in patients with PD-L1-negative tumors, the combination was superior to both agents as monotherapy [40]. Early results of a phase I study combining nivolumab and ipilimumab in 46 chemotherapy-naïve patients with NSCLC reported an ORR of 22 %, with an additional 33 % experiencing stable disease [4]. Responses were similar in patients with squamous and nonsquamous histologies (27 and 19 %), as well as in PD-L1-positive and PD-L1-negative tumors (19 and 14 %). Grade 3/4 treatment-related AEs occurred in 48 % of patients, and 3 patients dying of therapy-related complications (respiratory failure, bronchopulmonary hemorrhage, and toxic epidermal necrolysis). Early results of a phase 1 study evaluating pembrolizumab in combination with ipilimumab in patients with advanced, recurrent NSCLC found clinical responses in all doses groups among 11 evaluable patients [47]. A phase 1/2 trial (NCT01928394) of nivolumab both as monotherapy and in combination with ipilimumab in patients with SCLC, in addition to other solid tumors, is undergoing evaluation as well.

A phase I study evaluating the combination of MEDI-4736 and tremelimumab reported an overall response rate of 27 % and disease control rate of 48 % in 63 evaluable patients with PD-L1-negative tumors [3]. Toxicities included diarrhea, colitis, and elevated liver function tests. The dose combination selected for future studies (MEDI4736 20 mg every four weeks and tremelimumab 1 mg/kg every four weeks) was well tolerated, with grade 3/4 events in 4 of 22 patients at this dosing level.

2.5 Predictors of Response to Anti-PD-1 and Anti-PD-L1 Therapies

While agents targeting both PD-1 and PD-L1 have shown great promise in the treatment of NSCLC, only a subset of patients derives sustained clinical benefit, with response rates ranging from 16 to 23 % in unselected NSCLC patients across early trials [8, 9, 18, 20, 21, 57]. Thus, there is great interest in developing reliable predictors of response to therapy.

Tumor expression of PD-L1 by immunohistochemistry (IHC) has been studied as a potential biomarker of response to anti-PD-1/PD-L1. However, practical conclusions regarding the optimal use of PD-L1 as a predictive biomarker are complicated due to factors related to the assays and cell types used for measurement, as well as the biology of PD-L1 itself. Each of the major PD-1/PD-L1 antibodies in current trials has been developed with a unique companion diagnostic assay, each possessing individual performance specifications and thresholds for positivity. Definitions of PD-L1 “positivity” in various studies have ranged from ≥1 to ≥50 % of evaluated cells, which are generally tumor cells but in some cases may be tumor-infiltrating immune cells. By these various definitions, NSCLC specimens have been defined as PD-L1 “positive” in 13–70 % of samples; however, the degree of concordance across different testing platforms is unknown [39].

The clinical practicality of PD-L1 as a predictive biomarker is also unclear. Ideally, a biomarker should have either complete positive or negative value in predicting whether an

individual will respond to therapy. However, not all patients with PD-L1 positivity, even at its most stringent definitions, will respond to therapy, with ORR ranging from 16 to 83 % in PD-L1-"positive" patients, depending upon the drug and assay used. Conversely, there are patients who are PD-L1 "negative" who still respond to therapy, with ORR ranging from 3 to 20 % in various studies [39]. The dynamic nature of PD-L1 expression also indicates that it may be an imperfect biomarker. PD-L1 expression is stimulated by factors expressed within the tumor microenvironment, such as IFN-γ [66, 70], and biopsy specimens taken from a remote point in time may not accurately reflect expression levels present at the start of therapy. Whether the predictive value of PD-L1 expression depends upon histology is also unknown. For example, in Checkmate 057, a phase III study of nivolumab versus docetaxel in nonsquamous NSCLC, PD-L1 expression (defined at cutoffs of 1, 5, and 10 %) appeared to correlate positively with response and survival [48]. However, Checkmate 017, a phase III trial comparing nivolumab and docetaxel in previously treated patients with squamous NSCLC, found no correlation with PD-L1 expression and clinical response or survival [5]. Additionally, the optimal pattern of expression for predicting response is undetermined. PD-L1 assays commonly evaluate tumor cells, but expression on tumor-infiltrating immune cells also appears to be predictive [27]. In studies of atezolizumab, a PD-L1-directed antibody, both tumor cells and tumor-infiltrating immune cell populations were assessed for PD-L1 expressions [20]. Interestingly, although expression of PD-L1 by tumor cells and tumor-infiltrating immune cells could be found concurrently at low-to-moderate levels of expression, populations of tumor cells with high PD-L1 expression appeared to be exclusive of populations of tumor-infiltrating immune cells with high PD-L1 expression. Moreover, tumors with high PD-L1 expression in tumor cells (TC3) showed a dense desmoplastic and sclerotic tumor microenvironment with relatively scant immune infiltrates, while high PD-L1 expression in tumor-infiltrating immune cells (IC3) had an elevated frequency of immune infiltrates, as well a B- and NK-cell signatures. Although these two populations showed distinctive histopathologic characteristics, increased PD-L1 expression in either group was associated with an increased chance of response to atezolizumab therapy (OS HR, 0.47; PFS HR, 0.56 and ORR, 38 vs. 13 % in TC3 or IC3 patients) [65]. On the basis of these studies, PD-L1 expression appears to be a complicated, dynamic process, without a standard method of measurement at this point in time. Thus, while expression of PD-L1 may signify the general state of immune activity in the tumor microenvironment [62, 68] and is likely associated with clinical benefit of PD-1/PD-L1 directed therapy, its practical utility at this time remains to be determined.

Smoking status appears to have predictive value in several studies of PD-1 and PD-L1 agents. Response rates have been reported as higher among current or former smokers, as compared to nonsmokers [18, 26, 31]. It is thought that tumors related to a history of smoking may harbor a higher burden of somatic mutations [1, 11, 63], and a higher nonsynonymous mutation burden has been associated with improved responses, durable clinical benefit, and progression-free survival in NSCLC patients treated with pembrolizumab [54]. Measurement of various T cell-specific, antigen presentation-related, and IFN-γ signaling-related genes has been associated with response to pembrolizumab in melanoma, suggesting that responses are improved in the context of a preexisting interferon-mediated adaptive immune response [53].

2.6 Immune-Related Response Criteria

Immune checkpoint inhibitors have challenged traditional measures to evaluate clinical response. Early trials of ipilimumab in melanoma demonstrated that a subset of patients with apparent early progressive disease (increased tumor burden or appearance of new lesions) by traditional RECIST criteria ultimately showed clinical responses when followed over time. It was thus determined that confirmation of progression, as defined by an increased tumor burden of ≥25 % compared to nadir, must occur at two consecutive time points at least 4 weeks apart, in order for treatment to be determined a failure. These revised criteria for assessing therapeutic response have been termed immune-related response criteria [75] and are now commonly used in trials involving immune checkpoint inhibitors without chemotherapy.

2.7 Immune-Related Toxicities

Just as immunotherapies encompass a novel approach to tumor biology, the toxicities associated with these agents have created new challenges in the clinic. Unlike the toxicities of cytotoxic chemotherapy, side effects related to immune checkpoint inhibitors are autoimmune in nature. Generally, the incidence of immune-related toxicity is more frequent and more severe with ipilimumab as compared to anti-PD-1 and anti-PD-L1 agents; however, the immune-related toxicities can be life-threatening in either treatment class.

A pooled analysis of ipilimumab studies in melanoma found that approximately two-thirds of patients experienced an irAE, most of which were considered grade 1 and 2 [33]. Gastrointestinal and dermatologic toxicities were the most common class reported, but other significant immune-related toxicities included endocrine, hepatic, and neurological. Endocrine toxicity may be manifold and includes hypothyroidism, hyperthyroidism, hypophysitis, and adrenal insufficiency. Ipilimumab appears to have a relatively predictable kinetic profile with regard to toxicity, with timing of onset depending upon the organ system involved. Dermatologic irAEs tend to appear in the first 2–3 weeks of treatment, followed by gastrointestinal after 6–7 weeks, and endocrine occurring later, around 9 weeks [74]. Such guidelines are not absolute however, as late toxicity even after treatment discontinuation has been reported [12].

In contrast to anti-CTLA-4 therapy, toxicities related to anti-PD-1 and anti-PD-L1 agents are generally milder, but life-threatening presentations can occur. Commonly reported irAEs include dermatologic (rash, pruritus) and gastrointestinal (diarrhea, colitis), generally grade 1 or 2 in severity; other unique irAEs include hepatitis, hypophysitis, thyroiditis, and vitiligo [9, 56, 69]. Endocrine toxicity may be insidious, and monitoring of thyroid function during treatment may be helpful. Pneumonitis, while rare, is a unique toxicity of especial concern to lung cancer patients and may be associated more with anti-PD-1 agents than anti-PD-L1 therapies [36]. Most low-grade irAEs can be addressed with supportive measures and may not require therapy cessation. Management of grade 3/4 irAEs typically requires therapy discontinuation, as well as use of high dose intravenous steroids. A prolonged steroid taper after symptom resolution (up to 1 month) is generally advised [32].

3 Vaccine Therapy

Anticancer vaccines designed to elicit antigen-specific immune responses have been studied in lung cancer, albeit with less success than immune checkpoint inhibitors.

Melanoma-associated antigen-A3 (MAGE-A3) is an antigen expressed in approximately 35 % of NSCLCs, with higher levels of expression associated with more advanced disease and poor prognosis [24, 60]. The efficacy of the recombinant MAGE-A3 protein as a therapeutic vaccine was assessed in a phase II clinical trial of 182 resected early-stage NSCLC patients [73]. Patients were vaccinated with either the MAGE-A3 protein or placebo every three weeks for five cycles, followed by eight vaccinations every three months. No statistically significant improvement in time to progression, disease-free survival, or overall survival was seen with the vaccine therapy as compared to placebo. The MAGRIT trial was a phase III clinical trial of resected NSCLC patients selected for tumor expression of the MAGE-A3 protein [71]. Although the vaccine was well tolerated, the trial failed to meet its primary endpoint of improved disease-free survival with the addition of the vaccine [72].

Tecemotide (L-BLP25) is a liposome-based vaccine derived from the tandem repeat region of MUC1, a peptide expressed in NSCLC. Preclinical studies found that MUC1-directed immunotherapy successful induced a cellular immune response characterized by T cell proliferation and production of IFN-γ in a mouse model of NSCLC [78]. Correlation was also found between overall survival at one year and the presence of endogenous MUC1 antibodies in NSCLC patients [29]. The START trial enrolled 1513 patients with unrespectable NSCLC who had achieved either stable disease or an objective response after treatment with either concurrent or sequential chemoradiation. Patients were assigned to either tecemotide or placebo in a 2:1 ratio, with treatments occurring weekly for 8 weeks, and then every 6 weeks thereafter until progression. Although the trial failed to meet its endpoint of improved overall survival, a subgroup analysis of patients who received concurrent chemoradiation found an improvement in overall survival with tecemotide as compared to placebo (median OS 30.8 vs. 20.6 months; HR 0.78; $p = 0.016$) [10]. START2, a confirmatory trial of tecemotide in patients with stage III NSCLC after concurrent chemoradiation, is currently underway (NCT02049151).

Immune tolerance to tumor-associated antigens has been identified as a significant hurdle in the development of therapeutic lung cancer vaccines [50]. Although studies of lung cancer vaccines have been relatively lackluster, there is interest in combining cancer vaccines with immune checkpoint inhibitors, with the goal of inducing a stronger tumor-specific immune response. Whether vaccines may be a useful adjunct therapy to immune checkpoint inhibitors in the future remains to be determined.

4 Future Directions

The advent of effective immunotherapies for lung cancer bears potential for a new generation of promising treatments with novel toxicities. Early studies of immune checkpoint inhibitors as single agent therapy in NSCLC patients and in combination with chemotherapy in both NSCLC and SCLC patients have been encouraging. In patients that

respond to anti-PD-1 and PD-L1 therapy, responses appear to be both rapid and durable even beyond treatment discontinuation. However, many unanswered questions remain including the optimal patient population in which these agents will have benefit (PD-L1 positive or negative, specific molecular cohorts), the duration of therapy (one vs. two years), the sequence of therapy (prior to chemotherapy, in combination with chemotherapy or as maintenance therapy), and the appropriate combinations (chemotherapy, targeted therapy or combining anti-PD1 and anti-CTLA antibodies).

The identification of biomarkers to predict benefit from immune checkpoint therapy, as well as possibly more active combination regimens, are needed as only a subset of patients currently obtain the sustained responses that are desired. Additionally, although the toxicity profile of these agents is relatively favorable, the associated immune-related side effects present unique challenges in clinical management as they differ significantly from chemotherapy. Many phase III trials comparing anti-PD-1 and anti-PD-L1 antibodies both as monotherapy and in combination to standard first- and second-line therapies are ongoing. Given the manageable toxicity profile and potential for rapid, durable responses, it is expected that these novel therapies will continue to play a major role in the future of lung cancer treatment.

The significant cost of these agents is worth noting, with the average cost per patient listed at $12,500 per month for both nivolumab and pembrolizumab in 2015. Given that the greatest toxicity of these agents appears to be financial in nature, it is likely that immunotherapy, while providing great clinical advances for patients with NSCLC, will unfortunately add to growing fiscal challenges within the healthcare system as well. Development of economically sound pricing for these agents will be important considerations to optimize the positive impact they may have on outcome of lung cancer patients.

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