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Update on Immune Checkpoint Inhibitors in Lung Cancer

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Background: *The immune checkpoint proteins, including the B7/CD28 receptor superfamily, have become increasingly important targets for pharmacologic blockade. Several classes of new agents have impressive clinical activity, and their eventual approval for treatment of lung cancer seems likely.*
Methods: *This article discusses the current development of these agents, including the CTLA-4, PD-1, and PD-L1 inhibitory pathways, killer immunoglobulin receptor (KIR) inhibition, and other checkpoint proteins.*
Results: *Ipilimumab in combination with chemotherapy has exhibited encouraging results in small-cell and non–small-cell lung cancer alike. Reported phase I trials of the monoclonal antibodies nivolumab, MK-3475, MEDI4736, and MPDL3280A are demonstrating durable overall radiological response rates in the 20% to 25% range in lung cancer. This exceptional activity includes squamous lung cancers, a population historically bereft of significant therapeutic advances. Retrospective examination of tumor PD-L1 expression suggests that PD-L1 may eventually be evaluable as a predictive biomarker. Dual checkpoint blockade strategies, such as those combining anti-CTLA-4, anti-LAG-3, or anti-KIR, are being tested to increase the proportion and durability of tumor responses. Examination of acquired immune resistance and post-immunotherapy relapse strategies are underway.*
Conclusions: *These emerging antibodies hold great potential for the systemic control of epithelial cancers such as lung cancer.*

Introduction

Utilizing the immune system to eliminate cancer holds great potential. There is no medicine that can compare with the elaborate network of cellular interactions that the human body uses to repel foreign entities. The virtues of immunotherapy include its low toxicity profile, sustained surveillance activity, and ability to detect small numbers of tumor cells. Moreover, since memory B cells retain persistent activity, immune treatments may induce long-term remissions of cancer. Historically, immunotherapy has been viewed with skepticism and has had relatively little success in solid tumors. Specifically, no immune-related drugs have yet been approved for lung cancer in North America. However, several classes of new drugs appear to be active, and their impending approval for use in lung cancer seems likely. Reports of these initial successes have driven an explosion of immune drug development for this pervasive cancer.

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Lung Cancer: Driver Immunosuppressors

Similar to other epithelial tumors, lung cancer employs several methods to evade surveillance and elimination by the host immune system. For example, lung cancer cells undergo a slow process of immunoediting, wherein the precancerous cell gradually undergoes selective adaptation as it evolves to thwart immune surveillance.[1] Lung cancer cells also secrete soluble proteins that impede routine processing by antigen-presenting cells (APCs), including STAT-3, indoleamine 2,3-dioxygenase (IDO), transforming growth factor beta (TGF-β), and IL-10.[2-5] In addition, lung cancers may create a dense fibrotic stroma, which deters penetration by killer T cells altogether.[6,7] A substantial proportion of non–small-cell lung cancer (NSCLC) has downregulated major histocompatibility complex (MHC) class I expression.[8] MHC class I is a cell surface protein moiety loaded with cell-derived peptides required by T cells to recognize and destroy abnormal cells.[9,10] Likewise, lung tumors also induce aberrant expansion of CD4+ FoxP3+ regulatory T cells, which then inhibits cytotoxic T-cell and natural kill (NK) cell activity.[11] Myeloid-derived suppressor cells (MDSCs) are also upregulated by lung tumors, a process likely mediated by proinflammatory factors such as PGE2.[12] MDSCs cause reactive nitrosylation of antigens such as T-cell receptor (TCR), CD3, and CCR2, thus impeding T-cell function.[13,14] The term "driver immunosuppressors" has been proposed for this host of aberrations

Table 1. — Proposed "Driver Immunosuppressors" in Lung Cancer*

Phosphatidylserine externalization
Promotion of killer immunoglobulin receptor (KIR) 2DL1
Overexpression of B7 homolog 3
Induction of cytotoxic T-lymphocyte antigen-4 (CTLA-4)
Deletion or nitrosylation of tumor-associated antigen (TAA)
Loss of major histocompatibility complex (MHC) class I
Overexpression of programmed death ligand-1 (PD-L1)
Expression of N-glycolil-GM3 ganglioside
Upregulation of indoleamine 2,3 dioxygenase (IDO)

* In the future, each of these aberrations may be targeted with a specific drug therapy.

(Table 1). This phrase captures the idea that each immunosuppressive mechanism may be specific to a given patient's cancer, much like the canonical genetic targets like anaplastic-lymphoma kinase (ALK) rearrangement.

Given this myriad of immunosuppressive tools, it is of little wonder that traditional immunotherapy approaches have largely failed to eradicate lung cancer.[15] Nevertheless, the fruits of this scientific effort are beginning to be realized in lung cancer. This historic work has laid the foundation for a new class of biologic agents known as checkpoint inhibitors.

CTLA-4 Inhibition

The immune checkpoint pathway is an elaborate series of cellular interactions that prevents excessive effector activity by T cells under normal conditions. A principal part of this pathway is a cell surface receptor, called cytotoxic T-lymphocyte antigen-4 (CTLA-4, CD152). Once a cytotoxic T cell becomes active, it expresses CTLA-4 on its cell surface, which then competes with the costimulatory molecule CD28 for their mutually shared ligands, B7-1 (CD80) or B7-2 (CD86) on the APC. This "yin-yang" balance holds cytotoxic activity in check, while allowing T-cell function to proceed in a self-limited manner. Lung cancer can stimulate abnormal expression of CTLA-4 in T cells,[16] and these CTLA-4 aberrant T cells exhibit an anergic phenotype.[17] Thus, lung cancer cells may co-opt the CTLA-4 pathway to evade patrolling T cells. The introduction of monoclonal antibodies that inhibit CTLA-4 has achieved consistent and durable antitumor responses in several cancers, such as melanoma. Currently, two human monoclonal antibodies to CTLA-4 — tremelimumab and ipilimumab — are being tested in lung cancer.

Tremelimumab (formerly ticilimumab)

Tremelimumab (CP-675,206) is a human IgG2 monoclonal antibody with high affinity to CTLA-4. Initial activity of tremelimumab was originally shown in an open-label, phase II trial. This open-label trial randomized 87 patients with advanced NSCLC to treatment every 90 days or supportive care, following 4 cycles of platinum-based, first-line chemotherapy.[18] Although the drug did not prolong progression-free survival (PFS), 5% of participants achieved objective radiological responses. A 29-patient phase II trial in advanced mesothelioma also had a durable 7% radiologic partial response rate.[19] Tremelimumab is currently being tested in a randomized phase II trial for advanced mesothelioma (NCT01843374) and in combination with another checkpoint inhibitor for NSCLC (NCT01843374), as discussed later.

Ipilimumab

Ipilimumab (MDX-010) is a human IgG1 monoclonal antibody to CTLA-4, not unlike tremelimumab. A placebo-controlled multicenter phase II trial randomized patients 1:1:1 to 2 schedules of ipilimumab or to placebo during platinum-based chemotherapy for first-line treatment of advanced lung cancer.[20] A concurrent arm consisted of 4 cycles of chemotherapy with ipilimumab followed by 2 chemotherapy cycles with a placebo. A phased arm consisted of 2 chemotherapy cycles followed by 4 chemotherapy cycles with ipilimumab. Both arms received maintenance ipilimumab every 3 months until progression. The trial used a predefined immune-related (ir) primary end point called irPFS. The irPFS criteria accounts for the phenomena of "pseudo-progression." The puzzling occurrence on computed tomography of apparent tumor growth followed by sustained tumor regression is not uncommon with these checkpoint inhibitors.[21] This "pseudo-progression" phenomenon may be attributable to both delayed immune activity and initial peritumoral lymphocyte infiltration. Intriguingly, for the 204 NSCLC patients, the phased schedule seemed to slightly improve median irPFS (5.7 vs 4.6 months, hazard ratio [HR] = 0.82).[22] Lynch et al[20] reported a statistical significance level of $P = .05$ for irPFS improvement, but using a 1-sided alpha of 0.10, which is probably closer to .20 by usual standards.[23] Notable activity was observed in squamous lung cancers, a histology of NSCLC that has largely been bereft of important therapeutic advances.[24] A Japanese phase I trial demonstrated a radiological response rate of 60% in 10 evaluable NSCLC patients on the phased schedule of ipilimumab with platinium-based chemotherapy.[25] These encouraging results have led to a phase III trial for registration in squamous NSCLC using the phased ipilimumab schedule (NCT01285609). Overall survival is the primary end point, and the trial is currently completing accrual.[26] Similarly, a phase II trial included 130 extensive-disease small-cell lung cancer (ED-SCC) patients, and an identical modest improvement in median irPFS was achieved for the

phased schedule only. Specifically, the improvement was 6.4 vs 5.3 months (HR = 0.64, P = .03) with the nonstandard 1-sided alpha of 0.10.[27]

The observation of apparent benefit only in the phased schedule elicits conjecture why the drug may have particular activity only with this sequence. A proffered theory is that the stromal disruption and inflammatory milieu created by chemotherapy could be required for successful antigen presentation or T-cell effector activity.[28] A phase III trial to register ipilimumab in first-line ED-SCC is proceeding (NCT01450761) despite a challenging study population, and it has a primary end point of overall survival.[29] Of note, a multicenter phase II trial of ipilimumab compared with maintenance pemetrexed (NCT01471197) was terminated by the sponsor, presumably due to the success of other agents for nonsquamous NSCLC. An innovative institutional trial (NCT01820754) is testing preoperative ipilimumab in combination with neoadjuvant chemotherapy for resectable NSCLC and testing if tumor-infiltrating lymphocytes (TILs) correlate with response. Similar to tremelimumab, ipilimumab is also being tested in combination with other checkpoint inhibitors, as described later.

Based on these currently accruing trials, ipilimumab may become a helpful addition to our toolkit for therapy of advanced lung cancer. However, mitigation of adverse effects through supportive care will be critical. In comparison with the chemotherapy arm alone, a 14% to 17% higher incidence of all-cause grade 3 or 4 events was observed with ipilimumab. A substantial proportion of these events were directly related to autoimmune activity elicited by the drug, including one death due to epidermal necrolysis.[20]

PD-1 Inhibition

The programmed cell death-1 (PD-1) is another central interaction in the immune checkpoint pathway. Like CTLA-4, PD-1 is a surface receptor member of the B7-CD28 superfamily. It is expressed on many cell types, including activated T cells, B cells, NK cells, and host tissues.[30,31] As PD-1 docks with its ligand PD-L1 (B7-H1, CD274) on APCs, the interaction inhibits downstream NF-κB transcription and downregulates interferon (IFN)-δ secretion, ultimately inducing T-cell tolerance.[32] PD-1 also docks with PD-L2 (B7-DC, CD273) present on dendritic cells, although our understanding of the relevance of this interaction remains unclear.[33] In contrast to previous reports, PD-L2 appears to have inhibitory activity upon T cells similar to PD-L1.[34]

Numerous epithelial cancers may co-opt the PD-1 pathway, via aberrant cell-surface expression of PD-L1. This overexpressed PD-L1 protein induces T-cell anergy and circumvents the recognition and processing of their tumor antigens by APCs (Figure). Importantly, abnormal expression PD-L1 is identified in 19% to 100% of NSCLC tumors, depending in part on the antibody, histology, and technique reported.[35-38] In several reports, PD-L1 expression seems to be more commonly observed in sarcomatoid and adenocarcinoma subtypes of lung cancer, and it has been associated with poor prognosis.[35,36] Along these lines, TILs seem to be absent in PD-L1+ regions of tumors.[35] PD-L1 expression may be directly regulated by STAT-3 and appears to be further stimulated by immunosuppressive cytokines, such as IL-27.[37,38] Lung cancers thereby seem to protect themselves against killer T-cell elimination by adaptive upregulation of PD-L1. Of note, *PD-L1* messenger RNA expression appears to be no different in lung tumors compared with adjacent normal lung tissue, although it seems to be 3-fold higher in metastatic compared with early-stage disease.[39] Additionally, approximately 32% to 50% of lung cancers may express or cause transcription of B7 homologs 3 and 4 (B7-H3/B7-H4), which also mediate TIL suppression and immune evasion.[4,40-42] B7-H3 has at least 2 isoforms, 2IgB7-H3 and 4IgB7-H3, and may be a promising target for future drug development.[43]

Nivolumab

Inhibition by monoclonal antibody of PD-1 on CD8+ TILs within lung tumors is known to restore cytokine secretion and T-cell proliferation.[44] Nivolumab (BMS-936558) is a human monoclonal IgG4 antibody that essentially lacks detectable antibody-dependent cellular cytotoxicity (ADCC). In an early phase I trial of nivolumab, an objective response was observed in 22 patients (17%; 95% confidence interval [CI], 11%–25%) in a dose-expansion cohort of 129 previously treated patients with advanced NSCLC.[45] Six additional patients who had an unconventional immune-related response were not included. Moreover, the median duration of response was exceptional at 17 months. Although the median PFS in the cohort was 2.3 months and the median overall survival was 9.9 months, it seemed clear that those who responded had sustained benefit. Specifically, the 2-year overall survival rate was 24%, and many remained in remission after completing 96 weeks of continuous therapy. Moreover, little toxicity was observed, specifically, a 6.2% select grade 3/4 serious adverse event (AE) rate. While ipilimumab in lung cancer has reported gastrointestinal (GI) grade 3/4 AEs as high as 20%, this drug had a GI AE rate of only 2%. Nonetheless, 3 drug-related deaths occurred due to pneumonitis early in the trial course, which emphasizes the powerful mechanism of immune stimulation. Eight patients had any-grade drug-related pneumonitis. The precise signaling pathways of these autoimmune AEs remains unclear. With careful vigilance, pneumonitis can often be controlled early with corticosteroid administration.

Nivolumab has also been tested in combination with platinum-based chemotherapy for first-line NSCLC, with an objective response rate of 33% and a grade 3 or 4 AE rate of 49%, although these were in large part attributable to chemotherapy.[46] Phase III trials with prospective overall survival endpoints are currently underway (NCT01642004, NCT01673867). Additional trials are testing the combination of nivolumab with other checkpoint inhibitors (NCT01820754), a strategy that has reported synergistic activity in melanoma.[47] Moreover, a phase I trial is examining nivolumab alone and in combination with ipilimumab for small-cell lung cancer as well (NCT01928394).

In the above trials, patients generally receive anti–PD-1 antibody until progression for 1 to 2 years in total. If study participants achieve a durable response and then subsequently progress after cessation of therapy, there is an opportunity for rechallenge at the time of progression. This is based on early reports of patients who achieved an initial complete response to anti–PD-1, who were then re-treated with anti–PD-1 at the time of tumor recurrence.[48] Thus, it seems that immune suppression by ligands such as PD-L1 may creep back over time and that host T-cell function may be reestablished by resuming checkpoint blockade.

Current trials of nivolumab are requiring archival tissue for eligibility, with the ostensible intent of filing for registration selectively in the PD-L1+ subpopulation only if the primary endpoint is not achieved in the overall study population. Among the 129 patients with NSCLC treated on the original second-in-humans trial, tumor membrane PD-L1 expression was present in 31 of 63 evaluable biopsies. There was no association between PD-L1+ and histology, and objective responses were reported in 4 of 32 in PD-L1– and in 5 of 31 in PD-L1+.[49] Thus, in contrast to several

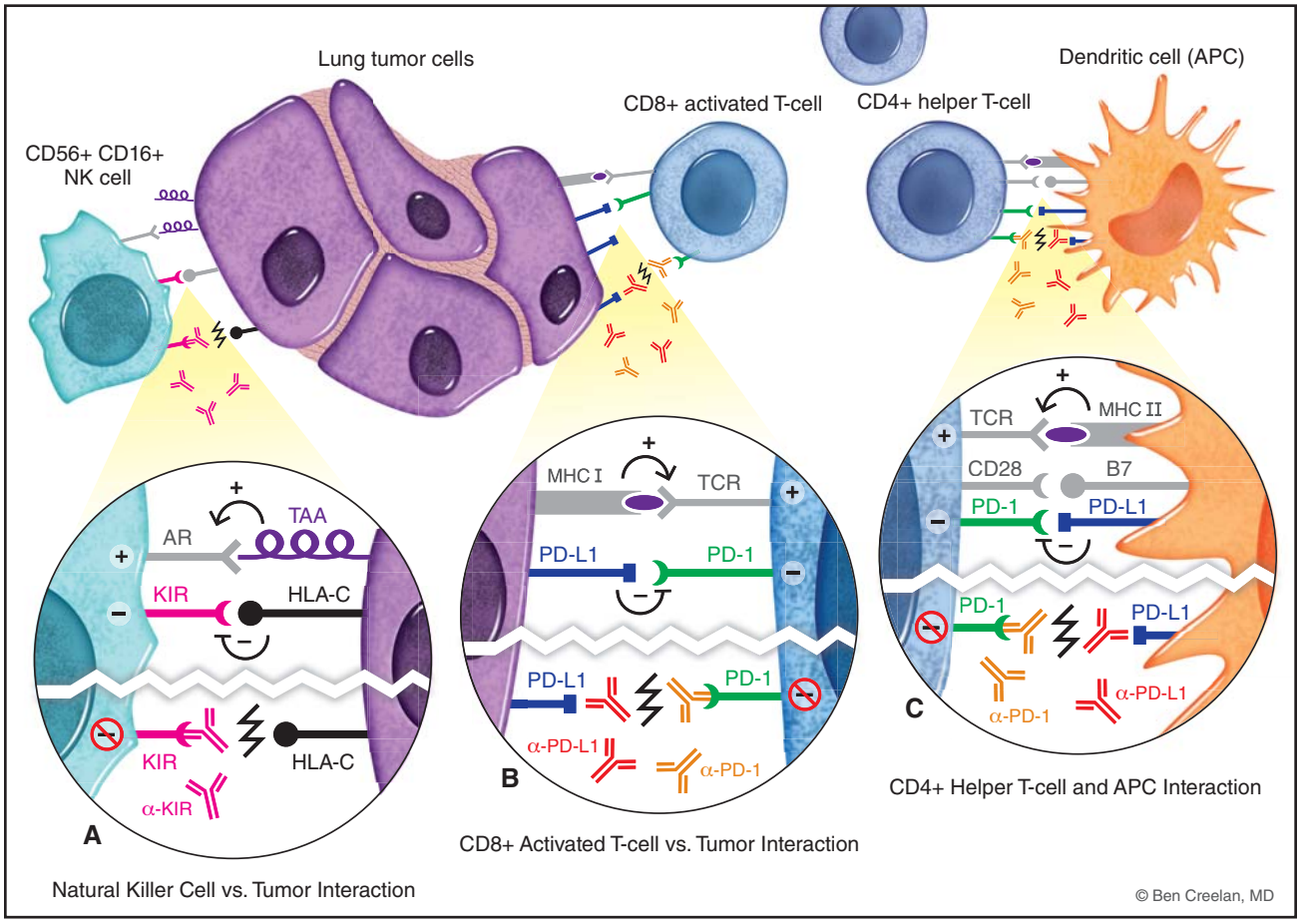

Figure. — Examples of checkpoint inhibition. (A) When patrolling natural killer (NK) cells encounter tumor cells, their activating receptor (AR) is stimulated by tumor-associated antigen (TAA). However, simultaneous interaction of inhibitory killer immunoglobulin receptors (KIRs) with tumor ligands, predominantly human leukocyte antigen (HLA-C), deactivates the NK cell. NK cell activity can be restored by the addition of monoclonal antibodies that bind to inhibitory KIRs, such as lirilumab, an IgG4 monoclonal antibody to KIR2DL1/2/3 (α-KIR). (B) CD8+ cytotoxic T cells become activated to kill tumors cells when their antigen-specific T-cell receptors (TCRs) bind major histocompatibility complex (MHC) class I on the tumor cell surface. However, tolerance occurs when the T-cell programmed-death receptor-1 (PD-1) interacts with its ligand, PD-L1, which is aberrantly expressed by the lung tumor cell. Infusion of monoclonal antibody to bind these proteins, as either α-PD-L1 (eg, MPDL3280A, MEDI4736) or α-PD-1 (eg nivolumab, MK-3475) abrogates this interaction, thus promoting effector T-cell–mediated rejection of tumor. (C) Dendritic cells are antigen-presenting cells (APCs) that load tumor peptides onto MHC class II protein and then present them to TCRs on CD4+ helper T-cells. A critical second signal is the binding of CD28 with B7-1/2 on the APC. After activation, the interaction of PD-1 with PD-L1 normally provides negative feedback by inducing helper T-cell anergy. This "off" signal can be blocked by α-PD-L1 or α-PD-1 antibody, thereby maintaining T-cell activity against cancer cells.

agents described below, PD-L1+ does not yet seem to have reliable value as a biomarker for nivolumab. Moreover, *EGFR* or *KRAS* mutation status does not appear to correlate with response rate.[50]

MK-3475

Similar in function to nivolumab, MK-3475 (formerly lambrolizumab) is a humanized IgG4 anti–PD-1 antibody that contains a mutation at C228P designed to prevent Fc-mediated ADCC. In an initial phase I report, no serious drug-related AEs were reported for this drug, and an unconfirmed partial response was noted in 1 patient with squamous NSCLC.[51] Although the principal registration track for this antibody has been in melanoma, NSCLC is now being pursued as well.[52] This is based on interim phase I data in 38 NSCLC patients as a single agent every 3 weeks, demonstrating an objective response rate of 24% using immune-related response criteria.[53] Only 53% of patients had drug-related AEs. The most common AEs were mild: fatigue, rash, and pruritus, at 16% each. One case of grade 3 pulmonary edema was reported, not unlike that reported with nivolumab. Pretreatment tumor PD-L1 expression by immunohistochemistry (IHC) was a statistically significant predictor of response. In evaluable archival samples, 6 of 9 PD-L1+ patients had responses compared with 1 of 24 PD-L1– patients. An important caveat is that the cut-point for PD-L1+ was not specified a priori. Median PFS was 9.7 weeks (95% CI, 7.6–17 weeks), and 2 of the 9 responders did progress at initial report. Median overall survival was 51 weeks. Based on these promising data, MK-3475 in PD-L1+ patients is currently being examined in the relapsed/refractory setting (NCT01905657) and in combination with first-line chemotherapy (NCT01840579). Additional anti–PD-1 antibodies are currently in clinical development.[52-55]

PD-L1 Inhibition

Another encouraging strategy is to inhibit PD-L1, the ligand for PD-1, on the tumor cell surface. One potential upside of this approach may be that it does not interfere with T-cell PD-1 receptor interaction with APCs via other ligands, such as B7-H2 (ICOS-L).[56] Theoretical downsides to selective PD-L1 inhibition include the potential ability of tumors to aberrantly upregulate expression of other inhibitory ligands for PD-1, such as B7-DC. Furthermore, drug-resistant clones may emerge after protein modification or mutation of the PD-L1 epitope.[35] Currently, 4 promising agents are targeting PD-L1. It remains uncertain whether PD-1 or PD-L1 inhibition, or the combination, may yield the most robust efficacy in solid tumors. Early success was seen in a phase I BMS-936559 trial, which reported an overall objective response rate of 12.5% in evaluable patients, including 5 of 49 NSCLC patients treated.[57] Although clinical development for BMS-936559 is closed, additional anti–PD-L1 agents are under investigation, as outlined below.

MPDL3280A

MPDL3280A (RG7446) is a human IgG1-kappa anti–PD-L1 monoclonal antibody that has a single amino acid substitution in its Fc region that normally docks with Fc receptors present on circulating immune cells. This deleted region is designed to avoid ADCC, thereby preventing inadvertent killing of bystander immune cells that also express PD-L1, such as activated T cells. A phase I trial of this agent included 85 patients with NSCLC and reported a 23% best overall response rate, with only 11% drug-related grade 3-4 AEs.[58,59] One grade 3 dyspnea and one grade 3 autoimmune diabetes was seen. No dose-limited toxicities were observed. Moreover, of the 53 patients with evaluable response and archival samples, 5 of the 6 participants with strong IHC (3+) baseline PD-L1 expression had responses. The majority of responses were observed within the first 14 weeks, and almost all responders completed 1 year of treatment without progression. Responses were reported in 11 of 43 former/current smokers compared to 1 of 10 never-smokers, and 8 of 27 with *KRAS* wild-type tumors compared with 1 of 10 *KRAS* mutant tumors. A 90-gene "immunochip" microarray, which includes genes putatively expressed in the PD-1 pathway, also appears to be associated with drug activity.[60] Consistent with its mechanism of action, activated HLA-DR+CD8+ T cells increased in peripheral blood after 2 weeks of treatment, although this finding did not correlate with radiological response. These results have prompted 2 phase II trials in NSCLC that select for patients who are PD-L1+ by the sponsor's proprietary PD-L1 IHC test (NCT01846416, NCT01903993).

Registration trials that use robust companion biomarkers are becoming increasingly important in oncology. Predictive markers allow treatment of populations with a larger effect size and larger benefit:risk ratio, thus permitting smaller trials and faster approval. Such predictive protein or gene-based classifiers appeal to patients, and they make sense from a economic and biologic perspective. Nonetheless, the role of PD-L1 as a predictive tumor biomarker continues to evolve over time. Small proportions of patients still achieve favorable responses to monoclonal antibodies such as nivolumab and MPDL3280A; despite the absence of PD-L1 expression by IHC. Moreover, published reports to date have not utilized robust predefined cut-points or independent external validation of methodology. Since the driver mechanisms of immune suppression are complex, it may be unrealistic to expect a simple PD-L1 IHC test to predict for drug response with ideal accuracy for routine

clinical use. A second drawback is that soluble cytokines such as IFN-α dramatically upregulate PD-L1 expression. Therefore, it may be that fresh tumor samples likely have better predictive value than the traditional baseline archived tissue that are often utilized in clinical practice.[61,62] Tissue-related problems such as these are being examined in ongoing prospective trials and large institutional retrospective series.

MEDI4736

MEDI4736 is another IgG1-kappa PD-L1 inhibitor that has shown promising early activity in NSCLC. Similar to MPDL3280A, it also has directed mutations in the Fc region that prevent binding to C1q and the Fcγ receptor, thus eliminating off-target complement-mediated cytotoxicity and ADCC. Interim results of a phase I trial reported no colitis or pneumonitis of any grade, with several durable remissions, including NSCLC patients.[63] This phase I trial is currently testing subjects prospectively for both baseline and post-treatment tumor PD-L1 expression with fresh biopsies (NCT01693562), and a second phase I trial testing the combination of MEDI4736 with tremelimumab is also accruing (NCT01975831).

AMP-224

Similar to described above, an alternative approach is to competitively block the PD-1 receptor, using a B7-DC-Fc fusion protein.[64] Some NSCLC patients were included in a first-in-man phase I trial of this fusion protein drug, called AMP-224. A dose-dependent reduction in PD-1–high TIL was observed at 4 hours and 2 weeks after drug administration.[65,66] Moreover, an increase in peripheral blood gene expression of the T-cell chemo-attractant CXCL9 was reported. Following the acquisition of the AMP-224 portfolio, this drug may also be developed to include treatment of select solid tumors such as NSCLC.

KIR Inhibition

Lirilumab

Industry and academic centers are also testing methods of blocking numerous other inhibitory checkpoint molecules to treat cancer. Killer cell immunoglobulin-like receptor (KIR) is a receptor on NK cells that downregulates NK cytotoxic activity. HLA class I allele-specific KIR receptors are expressed in cytolytic ($CD56^{dim}CD16^{+}$) NK cells, while $CD56^{bright}CD16^{-}$ NK subset lacks these KIRs.[67] Along these lines, inhibitory KIRs seem to be selectively expressed in the peritumoral NK cell infiltrate and thus seem to be a checkpoint pathway co-opted by tumors, similar to PD-L1.[68] KIRs have also been discovered to be important in mediating tolerance and reducing graft-vs-host disease in allogeneic stem cell transplantation.[69] The role of the KIR protein depends on its structure. An increased distribution of KIR2DL1 and its ligand HLA-C2 is reported in NSCLC, and a corresponding decrease in distribution of KIR2DL3 and its normal ligand HLA-C1.[70] Therefore, NSCLC seems to stimulate expression of the suppressive, high-affinity KIRs and their ligands.[71] This results in reduced NK activity, thus effectively protecting the cancer cells from NK-mediated destruction.[72] Fitting with this theory, the less suppressive KIR2DL3 phenotype is correlated with better response to treatment and more favorable survival in NSCLC.[73,74] Based on this knowledge, inhibition of specific KIRs should cause sustained in vivo activation of NK cells. Lirilumab (IPH2102), a fully human monoclonal antibody to KIR, in combination with nivolumab has demonstrated an early efficacy signal in preclinical models. A trial of nivolumab with lirilumab in human solid tumors is underway, including 32 NSCLC patients (NCT01714739).[75] A similar trial is also testing the combination of lirilumab with ipilimumab, accruing up to 20 NSCLC patients in a dose-expansion cohort (NCT01750580).[66]

Other Checkpoint Proteins

Urelumab

CD-137 (4-1BB) is a costimulatory checkpoint protein that can be pharmacologically activated using urelumab (BMS-663513), a fully human IgG4 monoclonal antibody. This agent has demonstrated promising activity in solid tumors.[76] This antibody activates a component of the tumor necrosis factor receptor expressed on the cell membrane of activated white blood cells. Reported toxicity, such as fatigue and transaminitis, was related primarily to induction of IFN-γ. Development in NSCLC has been halted by the sponsor, presumably because of other competing agents in the portfolio, although it is being tested in other cancers (NCT01471210).

LAG-3

Another checkpoint protein target is lymphocyte-activation gene 3 (LAG-3, CD223), a CD4-related inhibitory receptor coexpressed with PD-1 on tolerant TILs.[77,78] LAG-3 is also expressed on T-regs, and it suppresses APC activation by binding with MHC II. In animal models, inhibition of LAG-3 by a monoclonal antibody slows the growth of established tumors, and it causes synergistic tumor regression when combined with anti–PD-1 antibody.[79] Early-phase investigation of anti-LAG-3 monoclonal antibody (BMS-986016) alone and in combination with nivolumab is ongoing (NCT01968109).

Bavituximab

Phosphatidylserine (PS) is a phospholipid in normal cells that is translocated to the outer member surface during apoptosis, suppressing the excess immune ac-

Table 2. — Checkpoint Inhibitors Under Investigation for Lung Cancer

Drug Name	Drug Target	Phase I or II Result		Phase II or III Design				
		ORR in Lung	Sample Size	End Point	Control Arm	Sample Size	Population	Predictive Biomarker
BMS-936559[57]	IgG4 PD-L1 mAb	10.2%	49 of 286	None registered in NSCLC	N/A			
MEDI4736[63,84]	IgG1κ PD-L1 mAb	Not yet reported	90, some NSCLC	ORR				Contemporary PD-L1 IHC
MEDI4736 + tremelimumab[63]	IgG1κ PD-L1 mAb + IgG2 CTLA-4 mAb	Not yet reported	102, some NSCLC	ORR				Contemporary PD-L1 IHC
MK-3475 (lambrolizumab)[51,53]	IgG4 PD-1 mAb	24%*	38	ORR	None	90	First-line NSCLC dose expansion	Contemporary PD-L1 IHC
				OS	Docetaxel	920	Relapsed NSCLC	
MPDL3280A[58-60]	IgG1κ PD-L1 mAb	23%*	85	ORR	Docetaxel	180	Relapsed NSCLC	Archival PD-L1+ IHC, immunochip
Nivolumab (BMS-936558)[46,47]	IgG4 PD-1 mAb	17%	129 of 340	OS	Docetaxel	574	Relapsed non-SC	Archival PD-L1+ IHC
				ORR/OS	Docetaxel	264	Relapsed SC	
				ORR	None	100	Third-line SC	
Ipilimumab + chemotherapy[20]	IgG1κ CTLA-4 mAb	32%*	204	OS	Placebo	920	First-line SC chemotherapy with phased chemotherapy	N/A
Ipilimumab + chemotherapy[27]	IgG1κ CTLA-4 mAb	71%*	130	OS	Placebo	912	First-line ED-SCC, with phased chemotherapy	N/A
Nivolumab + ipilimumab[47]	IgG4 PD-1 mAb + IgG1κ CTLA-4 mAb	N/A	N/A	ORR	13 other arms	190	NSCLC	Archived PD-L1 IHC
Nivolumab ± ipilimumab[45]	IgG4 PD-1 mAb ± IgG1κ CTLA-4 mAb	N/A	160, some ED-SCC	No phase II/III yet registered in ED-SCC				Archived PD-L1 IHC
Pidilizumab (CT-011)[85]	IgG1 PD-1 mAb	N/A	0 of 17	None yet registered in NSCLC				N/A
AMP-224[65]	PD-L2/IgG1 fusion protein	N/A	11 of 44	None yet registered in NSCLC				PD-1+ TIL levels
Anti-OX40R antibody[86]	IgG CD134 mAb	N/A	30	None yet registered in NSCLC				N/A
Nivolumab + lirilumab (BMS-986015)[75]	PD-1 mAb + KIR IgG4	N/A	Some NSCLC of 150	None yet registered in NSCLC				TIL, PD-L1, HLA I by IHC
Bavituximab + chemotherapy[81-83]	Phosphatidylserine mAb	52%	49	ORR	None	25	First-line non-SC NSCLC	None to date

* Immune-related best overall response rate.

ED-SCC = extensive-disease small-cell lung cancer, IHC = immunohistochemistry, KIR = killer inhibitory receptor, mAb = monoclonal antibody, NSCLC = non–small cell lung cancer, N/A = not available, ORR = overall response rate, OS = overall survival, SC = squamous cell, TIL = tumor-infiltrating lymphocyte.

tivation that would otherwise occur during processing and clearance of decaying cell matter. Externalization of PS indirectly stimulates MDSCs and M2 macrophages, resulting in suppression of dendritic cell antigen presentation.[80] Like PD-L1, externalized PS is aberrantly expressed by some tumor cells and tumor-derived microvesicles. Thus, PS is believed to be exploited by tumors to prevent adaptive tumor immunity. Bavituximab (chimeric 3G4) is a chimeric IgG3 antibody against PS. A phase I single-agent trial in solid tumors demonstrated an acceptable safety profile, although no objective radiologic responses were seen.[81] A phase II trial testing bavituximab with first-line platinum-based chemotherapy in NSCLC reported a 52% overall response rate, with principal adverse effects consisting of pyrexia and diarrhea.[82] A small randomized trial suggested a benefit in overall survival compared to chemotherapy alone.[83] Additional trials of bavituximab in combination with chemotherapy in NSCLC are underway (NCT01323062).

Additional trials of checkpoint inhibitors are listed in Table 2.

N-Glycolil-GM3 Ganglioside Antibody

Racotumomab (formerly known as 1E10) is an anti-idiotype murine monoclonal antibody against the human monoclonal antibody for N-glycolil-GM3 ganglioside. An anti-idiotype antibody targets the idiotopes located in the variable region of another antibody, such as the antigen-binding site. These antibodies thereby stimulate the immune system, and thus may work similarly to tumor-associated antigens (TAAs). N-glycolil-GM2 is a glycolipid that is not usually expressed in human epithelial cells, but it is present within gangliosides, sulfatides, and other antigens expressed in some solid tumors. It appears to correlate with survival and suppression of immune activity in NSCLC, among other cancers.[87-89] On the basis of a small trial reporting a few adverse effects, racotumomab received controversial approval in Argentina and Cuba for the treatment of advanced NSCLC. Currently, an international phase III trial (NCT01460472) of racotumomab is underway in advanced NSCLC with a planned accrual of 1,018 participants.[90] The primary end point is overall survival; however, the interpretation of response rate or benefit may be confounded by its open-label design.

Checkpoint Inhibitors in Combination With Vaccines

Despite traditional pessimism, cancer vaccines may be more relevant now than at any prior phase of oncology research. Frequently, vaccines displayed excellent activity in priming and expanding TAA-specific T cells, but in hindsight these efforts were invariably hampered by an unfavorably immunosuppressive tumor microenvironment. The current triumph of monoclonal antibodies that circumvent immunosuppression indicates that vaccines need to tested again in combination trials.

Driver Immunosuppressive Mechanisms: New Druggable Targets for NSCLC

Several mechanisms exist whereby tumors evade rejection by the immune system, as outlined in Table 1. It is monumental that inhibition of a single protein, PD-1, is enough to induce robust cancer remission in relapsed lung cancers. Like the somatic rearrangements revealed by sequencing tumors for genetic changes, a specific "driver" immunosuppressive pathway may be responsible for cancer cell proliferation. Not unlike the exquisite specificity of gefitinib or crizotinib, specific inhibition of the driver immunosuppressor likely will stop cancer cell growth for a subset of tumors.[91,92] Like PD-1, other immune escape aberrations may be potentially targeted with a specific drug therapy. Targeting these drivers could similarly yield durable tumor regressions in a specific subset of subjects. Therefore, immunotherapy has 2 principal challenges ahead of it. The first is to effectively bypass the driver immune escape mechanism. The second is to determine which driver immunosuppressor is active within an individual patient tumor, allowing for personalized therapy.

Conclusions

Some of the studies described are expected to yield a dramatic impact in treatment for patients with lung cancer. Statistically significant reports in oncology are often taken at face value, without critically probing their context and methodology.[93] In this regard, immunotherapy is receiving particular interest due to its favorable benefit:risk ratio and durable activity. Traditionally, the mainstay of systemic treatment of advanced lung cancer has been direct inhibition of tumor cell growth via small-molecule inhibitors or chemotherapy. Eventual relapse or progression was accepted as essentially inevitable, and increasingly less important end points had been adopted, such as time to progression.[94,95] Based on this dismal outlook, several expensive cancer treatments have been approved, for essentially marginal gains in progression-free survival.[96,97] Rarely have such systemic therapies translated into cures or durable remissions in disseminated solid tumors.[98] By contrast, the advent of immune therapies holds the potential to raise the tail of the survival curve.[99] Along these lines, the era of giant registration trials boasting median improvements of a few weeks may be all but over. Thus, effective immunotherapy may transform our expectations regarding what cancer treatment is, and we look forward to this promising future.

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