

## 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.

### 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.<sup>1</sup> 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- $\beta$ ), and IL-10.<sup>2-5</sup> In addition, lung cancers may create a dense fibrotic stroma, which deters penetration by killer T cells altogether.<sup>6,7</sup> A substantial proportion of non-small-cell lung cancer (NSCLC) has downregulated major histocompatibility complex (MHC) class I expression.<sup>8</sup> MHC class I is a cell surface protein moiety loaded with cell-derived peptides required by T cells to recognize and destroy abnormal cells.<sup>9,10</sup> Likewise, lung tumors also induce aberrant expansion of CD4<sup>+</sup> FoxP3<sup>+</sup> regulatory T cells, which then inhibits cytotoxic T-cell and natural kill (NK) cell activity.<sup>11</sup> Myeloid-derived suppressor cells (MDSCs) are also upregulated by lung tumors, a process likely mediated by proinflammatory factors such as PGE2.<sup>12</sup> MDSCs cause reactive nitrosylation of antigens such as T-cell receptor (TCR), CD3, and CCR2, thus impeding T-cell function.<sup>13,14</sup> The term “driver immunosuppressors” has been proposed for this host of aberrations

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Submitted October 24, 2013; accepted November 8, 2013.

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**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.<sup>15</sup> 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,<sup>16</sup> and these CTLA-4 aberrant T cells exhibit an anergic phenotype.<sup>17</sup> 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 humanized 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.<sup>18</sup> 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.<sup>19</sup> 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.<sup>20</sup> 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.<sup>21</sup> 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).<sup>22</sup> Lynch et al<sup>20</sup> 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.<sup>23</sup> Notable activity was observed in squamous lung cancers, a histology of NSCLC that has largely been bereft of important therapeutic advances.<sup>24</sup> A Japanese phase I trial demonstrated a radiological response rate of 60% in 10 evaluable NSCLC patients on the phased schedule of ipilimumab with platinum-based chemotherapy.<sup>25</sup> 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.<sup>26</sup> 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.<sup>27</sup>

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.<sup>28</sup> 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.<sup>29</sup> 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.<sup>20</sup>

## 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.<sup>30,31</sup> As PD-1 docks with its ligand PD-L1 (B7-H1, CD274) on APCs, the interaction inhibits downstream NF- $\kappa$ B transcription and downregulates interferon (IFN)- $\delta$  secretion, ultimately inducing T-cell tolerance.<sup>32</sup> 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.<sup>33</sup> In contrast to previous reports, PD-L2 appears to have inhibitory activity upon T cells similar to PD-L1.<sup>34</sup>

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.<sup>35-38</sup> 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.<sup>35,36</sup> Along these lines, TILs seem to be absent in PD-L1+ regions of tumors.<sup>35</sup> PD-L1 expression may be directly regulated by STAT-3 and appears to be further stimulated by immunosuppressive cytokines, such as IL-27.<sup>37,38</sup> 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.<sup>39</sup> 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.<sup>4,40-42</sup> B7-H3 has at least 2 isoforms, 2IgB7-H3 and 4IgB7-H3, and may be a promising target for future drug development.<sup>43</sup>

## Nivolumab

Inhibition by monoclonal antibody of PD-1 on CD8+ TILs within lung tumors is known to restore cytokine secretion and T-cell proliferation.<sup>44</sup> Nivolumab (BMS-936558) is a humanized 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.<sup>45</sup> 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.<sup>46</sup> 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.<sup>47</sup> 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.<sup>48</sup> 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+.<sup>49</sup> Thus, in contrast to several

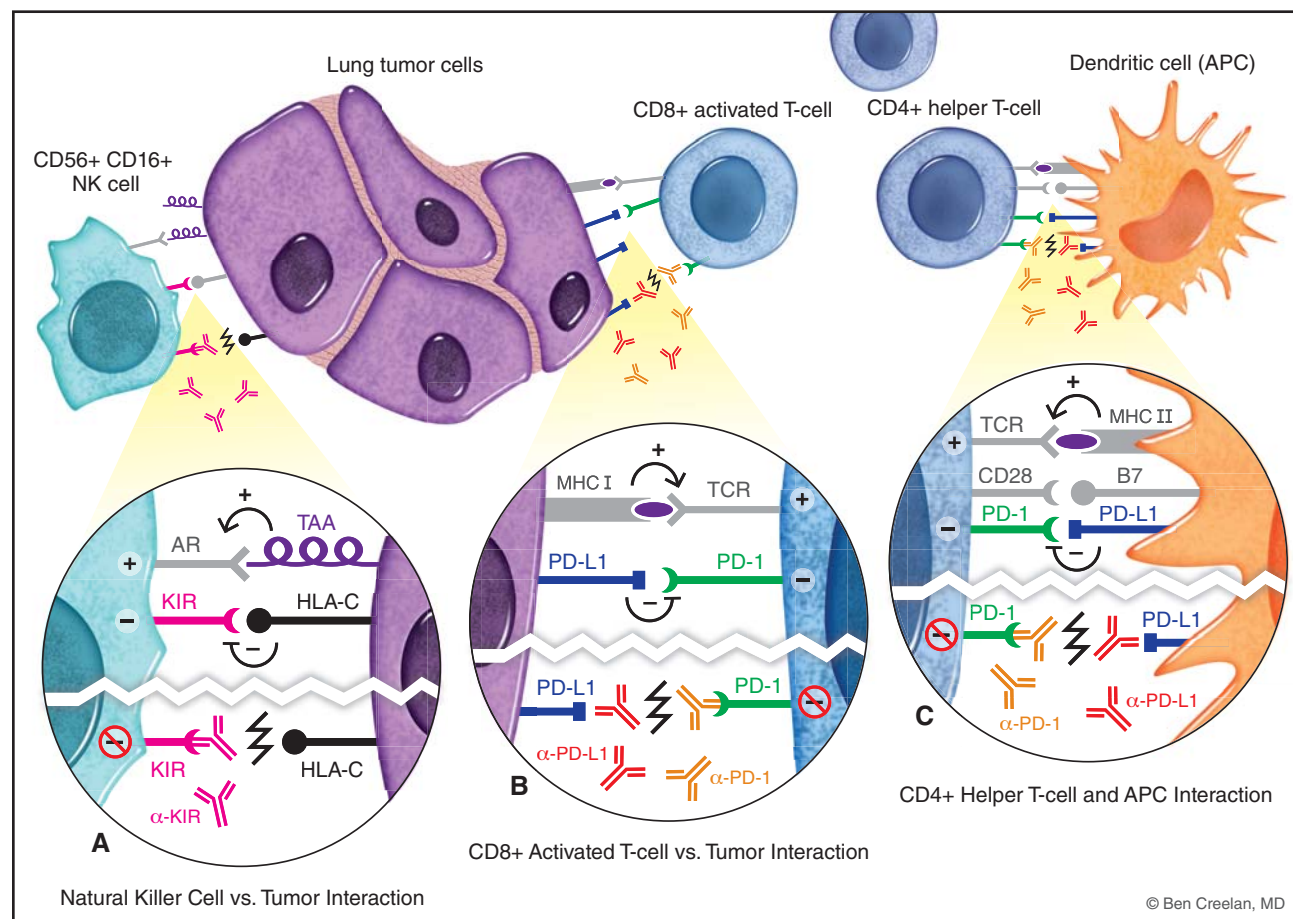


Figure. — Examples of checkpoint inhibition. (A) When patrolling natural killer (NK) cells encounter tumor cells, their activating receptor (AR) is stimulated by tumor-associated antigen (TAA). However, simultaneous interaction of inhibitory killer immunoglobulin receptors (KIRs) with tumor ligands, 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.<sup>50</sup>

### **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.<sup>51</sup> Although the principal registration track for this antibody has been in melanoma, NSCLC is now being pursued as well.<sup>52</sup> 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.<sup>53</sup> 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.<sup>52-55</sup>

### **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).<sup>56</sup> 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.<sup>35</sup> 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.<sup>57</sup> 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.<sup>58,59</sup> 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.<sup>60</sup> 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 an 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- $\alpha$  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.<sup>61,62</sup> 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 Fc $\gamma$  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.<sup>63</sup> 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.<sup>64</sup> 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.<sup>65,66</sup> 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<sup>dim</sup>CD16<sup>+</sup>) NK cells, while CD56<sup>bright</sup>CD16<sup>-</sup> NK subset lacks these KIRs.<sup>67</sup> 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.<sup>68</sup> KIRs have also been discovered to be important in mediating tolerance and reducing graft-vs-host disease in allogeneic stem cell transplantation.<sup>69</sup> 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.<sup>70</sup> Therefore, NSCLC seems to stimulate expression of the suppressive, high-affinity KIRs and their ligands.<sup>71</sup> This results in reduced NK activity, thus effectively protecting the cancer cells from NK-mediated destruction.<sup>72</sup> Fitting with this theory, the less suppressive KIR2DL3 phenotype is correlated with better response to treatment and more favorable survival in NSCLC.<sup>73,74</sup> 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).<sup>75</sup> A similar trial is also testing the combination of lirilumab with ipilimumab, accruing up to 20 NSCLC patients in a dose-expansion cohort (NCT01750580).<sup>66</sup>

## **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.<sup>76</sup> 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- $\gamma$ . 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.<sup>77,78</sup> 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.<sup>79</sup> 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 <sup>57</sup>	IgG4 PD-L1 mAb	10.2%	49 of 286	None registered in NSCLC	N/A			
MEDI4736 <sup>63,84</sup>	IgG1κ PD-L1 mAb	Not yet reported	90, some NSCLC	ORR				Contemporary PD-L1 IHC
MEDI4736 + tremelimumab <sup>63</sup>	IgG1κ PD-L1 mAb + IgG2 CTLA-4 mAb	Not yet reported	102, some NSCLC	ORR				Contemporary PD-L1 IHC
MK-3475 (lambrolizumab) <sup>51,53</sup>	IgG4 PD-1 mAb	24%*	38	ORR	None	90	First-line NSCLC dose expansion	Contemporary PD-L1 IHC
				OS	Docetaxel	920	Relapsed NSCLC	
MPDL3280A <sup>58-60</sup>	IgG1κ PD-L1 mAb	23%*	85	ORR	Docetaxel	180	Relapsed NSCLC	Archival PD-L1+ IHC, immunochip
Nivolumab (BMS-936558) <sup>46,47</sup>	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 <sup>20</sup>	IgG1κ CTLA-4 mAb	32%*	204	OS	Placebo	920	First-line SC chemotherapy with phased chemotherapy	N/A
Ipilimumab + chemotherapy <sup>27</sup>	IgG1κ CTLA-4 mAb	71%*	130	OS	Placebo	912	First-line ED-SCC, with phased chemotherapy	N/A
Nivolumab + ipilimumab <sup>47</sup>	IgG4 PD-1 mAb + IgG1κ CTLA-4 mAb	N/A	N/A	ORR	13 other arms	190	NSCLC	Archived PD-L1 IHC
Nivolumab ± ipilimumab <sup>45</sup>	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) <sup>85</sup>	IgG1 PD-1 mAb	N/A	0 of 17	None yet registered in NSCLC				N/A
AMP-224 <sup>65</sup>	PD-L2/IgG1 fusion protein	N/A	11 of 44	None yet registered in NSCLC				PD-1+ TIL levels
Anti-OX40R antibody <sup>86</sup>	IgG CD134 mAb	N/A	30	None yet registered in NSCLC				N/A
Nivolumab + lirilumab (BMS-986015) <sup>75</sup>	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 <sup>81-83</sup>	Phosphatidylserine mAb	52%	49	ORR	None	25	First-line non-SC NSCLC	None to date
<p>* Immune-related best overall response rate.</p> <p>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.</p>								



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.<sup>80</sup> 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.<sup>81</sup> 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.<sup>82</sup> A small randomized trial suggested a benefit in overall survival compared to chemotherapy alone.<sup>83</sup> 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-idiotypic murine monoclonal antibody against the human monoclonal antibody for N-glycolil-GM3 ganglioside. An anti-idiotypic 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.<sup>87-89</sup> 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.<sup>90</sup> 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 immuno-

suppressive tumor microenvironment. The current triumph of monoclonal antibodies that circumvent immunosuppression indicates that vaccines need to be 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.<sup>91,92</sup> 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.<sup>93</sup> 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.<sup>94,95</sup> Based on this dismal outlook, several expensive cancer treatments have been approved, for essentially marginal gains in progression-free survival.<sup>96,97</sup> Rarely have such systemic therapies translated into cures or durable remissions in disseminated solid tumors.<sup>98</sup> By contrast, the advent of immune therapies holds the potential to raise the tail of the survival curve.<sup>99</sup> 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.



## References

1. Matsushita H, Vesely MD, Koboldt DC, et al. Cancer exome analysis reveals a T-cell-dependent mechanism of cancer immunoediting. *Nature*. 2012;482(7385):400-404.
2. Derniame S, Vignaud JM, Faure GC, et al. Alteration of the immunological synapse in lung cancer: a microenvironmental approach. *Clin Exp Immunol*. 2008;154(1):48-55.
3. Kida H, Ihara S, Kumanogoh A. Involvement of STAT3 in immune evasion during lung tumorigenesis. *Oncoimmunology*. 2013;2(1):e22653.
4. Schneider T, Hoffmann H, Dienemann H, et al. Non-small cell lung cancer induces an immunosuppressive phenotype of dendritic cells in tumor microenvironment by upregulating B7-H3. *J Thorac Oncol*. 2011;6(7):1162-1168.
5. Sterlacci W, Wolf D, Savic S, et al. High transforming growth factor- $\beta$  expression represents an important prognostic parameter for surgically resected non-small cell lung cancer. *Hum Pathol*. 2012;43(3):339-349.
6. Salmon H, Donnadieu E. Within tumors, interactions between T cells and tumor cells are impeded by the extracellular matrix. *Oncoimmunology*. 2012;1(6):992-994.
7. Salmon H, Franciszewicz K, Damotte D, et al. Matrix architecture defines the preferential localization and migration of T cells into the stroma of human lung tumors. *J Clin Invest*. 2012;122(3):899-910.
8. Ramnath N, Tan D, Li Q, et al. Is downregulation of MHC class I antigen expression in human non-small cell lung cancer associated with prolonged survival? *Cancer Immunol Immunother*. 2006;55(8):891-899.
9. Lin A, Zhu CC, Chen HX, et al. Clinical relevance and functional implications for human leucocyte antigen-g expression in non-small-cell lung cancer. *J Cell Mol Med*. 2010;14(9):2318-2329.
10. Hanagiri T, Shigematsu Y, Kuroda K, et al. Prognostic implications of human leukocyte antigen class I expression in patients who underwent surgical resection for non-small-cell lung cancer. *J Surg Res*. 2013;181(2):e57-e63.
11. Woo EY, Yeh H, Chu CS, et al. Cutting edge: regulatory T cells from lung cancer patients directly inhibit autologous T cell proliferation. *J Immunol*. 2002;168(9):4272-4276.
12. Dieu-Nosjean MC, Antoine M, Danel C, et al. Long-term survival for patients with non-small-cell lung cancer with intratumoral lymphoid structures. *J Clin Oncol*. 2008;26(27):4410-4417.
13. Lesokhin AM, Hohl TM, Kitano S, et al. Monocytic CCR2+ myeloid-derived suppressor cells promote immune escape by limiting activated CD8 T-cell infiltration into the tumor microenvironment. *Cancer Res*. 2012;72(4):876-886.
14. Nagaraj S, Youn JI, Gabrilovich DI. Reciprocal relationship between myeloid-derived suppressor cells and T cells. *J Immunol*. 2013;191(1):17-23.
15. Kelly RJ, Giaccone G. Lung cancer vaccines. *Cancer J*. 2011;17(5):302-308.
16. Erfani N, Mehrabadi SM, Ghayumi MA, et al. Increase of regulatory T cells in metastatic stage and CTLA-4 over expression in lymphocytes of patients with non-small cell lung cancer (NSCLC). *Lung Cancer*. 2012;77(2):306-311.
17. Li L, Chao QG, Ping LZ, et al. The prevalence of FOXP3+ regulatory T-cells in peripheral blood of patients with NSCLC. *Cancer Biother Radiopharm*. 2009;24(3):357-367.
18. Zatloukal P, Heo DS, Park K, et al. Randomized phase II clinical trial comparing tremelimumab (CP-675,206) with best supportive care (BSC) following first-line platinum-based therapy in patients (pts) with advanced non-small cell lung cancer (NSCLC). *J Clin Oncol*. 2009;27(15S):8071. Abstract.
19. Calabrò L, Morra A, Fonsatti E, et al. Tremelimumab for patients with chemotherapy-resistant advanced malignant mesothelioma: an open-label, single-arm, phase 2 trial. *Lancet Oncol*. 2013;14(11):1104-1111.
20. Lynch TJ, Bondarenko I, Luft A, et al. Ipilimumab in combination with paclitaxel and carboplatin as first-line treatment in stage IIIB/IV non-small-cell lung cancer: results from a randomized, double-blind, multicenter phase II study. *J Clin Oncol*. 2012;30(17):2046-2054.
21. Oxnard GR, Morris MJ, Hodi FS, et al. When progressive disease does not mean treatment failure: reconsidering the criteria for progression. *J Natl Cancer Inst*. 2012;104(20):1534-1541.
22. Wolchok JD, Hoos A, O'Day S, et al. Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. *Clin Cancer Res*. 2009;15(23):7412-7420.
23. Good PI, Hardin JW. *Common Errors in Statistics (and How to Avoid Them)*. Hoboken, NJ: John Wiley & Sons; 2012.
24. Drilon A, Rekhtman N, Ladanyi M, et al. Squamous-cell carcinomas of the lung: emerging biology, controversies, and the promise of targeted therapy. *Lancet Oncol*. 2012;13(10):e418-e426.
25. Nokihara H. Phase I study of ipilimumab in combination with paclitaxel/carboplatin in patients with non-small cell lung cancer. Presented at: IASLC 15th World Conference on Lung Cancer; October 2013; Sydney, Australia; P2.11-040.
26. Genova C, Rijavec E, Barletta G, et al. Ipilimumab (MDX-010) in the treatment of non-small cell lung cancer. *Expert Opin Biol Ther*. 2012;12(7):939-948.
27. Reck M, Bondarenko I, Luft A, et al. Ipilimumab in combination with paclitaxel and carboplatin as first-line therapy in extensive-disease small-cell lung cancer: results from a randomized, double-blind, multicenter phase 2 trial. *Ann Oncol*. 2013;24(1):75-83.
28. Liu H, Zhang T, Ye J, et al. Tumor-infiltrating lymphocytes predict response to chemotherapy in patients with advanced non-small cell lung cancer. *Cancer Immunol Immunother*. 2012;61(10):1849-1856.
29. Reck M. What future opportunities may immuno-oncology provide for improving the treatment of patients with lung cancer? *Ann Oncol*. 2012;23(suppl 8):viii28-viii34.
30. Agata Y, Kawasaki A, Nishimura H, et al. Expression of the PD-1 antigen on the surface of stimulated mouse T and B lymphocytes. *Int Immunol*. 1996;8(5):765-772.
31. Sauce D, Almeida JR, Larsen M, et al. PD-1 expression on human CD8 T cells depends on both state of differentiation and activation status. *AIDS*. 2007;21(15):2005-2013.
32. Liang SC, Latchman YE, Buhlmann JE, et al. Regulation of PD-1, PD-L1, and PD-L2 expression during normal and autoimmune responses. *Eur J Immunol*. 2003;33(10):2706-2716.
33. Wang S, Bajorath J, Flies DB, et al. Molecular modeling and functional mapping of B7-H1 and B7-DC uncouple costimulatory function from PD-1 interaction. *J Exp Med*. 2003;197(9):1083-1091.
34. Pfistershammer K, Klausner C, Pickl WF, et al. No evidence for dualism in function and receptors: PD-L2/B7-DC is an inhibitory regulator of human T cell activation. *Eur J Immunol*. 2006;36(5):1104-1113.
35. Konishi J, Yamazaki K, Azuma M, et al. B7-H1 expression on non-small cell lung cancer cells and its relationship with tumor-infiltrating lymphocytes and their PD-1 expression. *Clin Cancer Res*. 2004;10(15):5094-5100.
36. Mu CY, Huang JA, Chen Y, et al. High expression of PD-L1 in lung cancer may contribute to poor prognosis and tumor cells immune escape through suppressing tumor infiltrating dendritic cells maturation. *Med Oncol*. 2011;28(3):682-688.
37. Hirahara K, Ghoreschi K, Yang XP, et al. Interleukin-27 priming of T cells controls IL-17 production in trans via induction of the ligand PD-L1. *Immunity*. 2012;36(6):1017-1030.
38. Wölfe SJ, Strebovsky J, Bartz H, et al. PD-L1 expression on tolerogenic APCs is controlled by STAT-3. *Eur J Immunol*. 2011;41(2):413-424.
39. Sasaki H, Suzuki A, Shitara M, et al. PD-L1 gene expression in Japanese lung cancer patients. *Biomed Rep*. 2013;1(1):93-96.
40. Chen C, Shen Y, Qu QX, et al. Induced expression of B7-H3 on the lung cancer cells and macrophages suppresses T-cell mediating anti-tumor immune response. *Exp Cell Res*. 2013;319(1):96-102.
41. Zhang G, Xu Y, Lu X, et al. Diagnosis value of serum B7-H3 expression in non-small cell lung cancer. *Lung Cancer*. 2009;66(2):245-249.
42. Sun Y, Wang Y, Zhao J, et al. B7-H3 and B7-H4 expression in non-small-cell lung cancer. *Lung Cancer*. 2006;53(2):143-151.
43. Zhou YH, Chen YJ, Ma ZY, et al. 4IgB7-H3 is the major isoform expressed on immunocytes as well as malignant cells. *Tissue Antigens*. 2007;70(2):96-104.
44. Zhang Y, Huang S, Gong D, et al. Programmed death-1 upregulation is correlated with dysfunction of tumor-infiltrating CD8+ T lymphocytes in human non-small cell lung cancer. *Cell Mol Immunol*. 2010;7(5):389-395.
45. Brahmer J. Nivolumab (anti-PD-1; BMS-936558; ONO-4538) in patients with non-small cell lung cancer (NSCLC): overall survival and long-term safety in a phase 1 trial. Presented at: IASLC 15th World Conference on Lung Cancer; October 2013; Sydney, Australia. MO18.03.
46. Rizvi N. A phase I study of nivolumab (anti-PD-1; BMS-936558, ONO-4538) plus platinum-based doublet chemotherapy (PT-doublet) in chemotherapy-naïve non-small cell lung cancer (NSCLC) patients (pts). *J Clin Oncol*. 2013;31(suppl; abstr 8072).
47. Wolchok JD, Kluger H, Callahan MK, et al. Nivolumab plus ipilimumab in advanced melanoma. *N Engl J Med*. 2013;369:122-133.
48. Lipson EJ, Sharfman WH, Drake CG, et al. Durable cancer regression off-treatment and effective reinduction therapy with an anti-PD-1 antibody. *Clin Cancer Res*. 2013;19(2):462-468.
49. Antonia S. Association of tumor PD-L1 expression and immune biomarkers with clinical activity in patients with non-small cell lung cancer treated with nivolumab. Presented at: IASLC 15th World Conference on Lung Cancer; October 2013; Sydney, Australia. P2.11-035.
50. Gettinger SH. Efficacy of nivolumab in patients with previously treated advanced non-small cell lung cancer: subpopulation response analysis in a phase I trial. Presented at: IASLC 15th World Conference on Lung Cancer; October 2013; Sydney, Australia. P2.11-038.
51. Patnaik A, Kang SP, Tolcher AW, et al. Phase I study of MK-3475 (anti-PD-1 monoclonal antibody) in patients with advanced solid tumors. *J Clin Oncol*. 2012;30(suppl):2512. Abstract.
52. Hamid O, Robert C, Daud A, et al. Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma. *N Engl J Med*. 2013;369(2):134-144.
53. Garon E. Preliminary clinical safety and activity of MK-3475 monotherapy for the treatment of previously treated patients with non-small cell lung cancer. Presented at: IASLC 15th World Conference on Lung Cancer; October 2013; Sydney, Australia. MO18.02.
54. Beck A, Wurch T, Reichert JM. 6th Annual European Antibody Congress MABs. 2011(2):111-132.

55. Mkrtychyan M, Najjar YG, Raulfs EC, et al. Anti-PD-1 synergizes with cyclophosphamide to induce potent anti-tumor vaccine effects through novel mechanisms. *Eur J Immunol*. 2011;41(10):2977-2986.
56. Bennett F, Luxenberg D, Ling V, et al. Program death-1 engagement upon TCR activation has distinct effects on costimulation and cytokine-driven proliferation: attenuation of ICOS, IL-4, and IL-21, but not CD28, IL-7, and IL-15 responses. *J Immunol*. 2003;170(2):711-718.
57. Brahmer JR, Tykodi SS, Chow LQ, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. *N Engl J Med*. 2012;366(26):2455-2465.
58. Herbst R, Gordon MS, Fine GD, et al. A study of MPDL3280A, an engineered PD-L1 antibody in patients with locally advanced or metastatic tumors. *J Clin Oncol*. 2013;31(suppl):3000. Abstract.
59. Horn L. An analysis of the relationship of clinical activity to baseline EGFR status, PD-L1 expression and prior treatment history in patients with non-small cell lung cancer (NSCLC) following PD-L1 blockade with MPDL3280A (anti-PDL1). Presented at: IASLC 14th World Conference on Lung Cancer; July 2012; Amsterdam, the Netherlands. MO18.01.
60. Powderly JD, Koeppen H, Hodi S, et al. Biomarkers and associations with the clinical activity of PD-L1 blockade in a MPDL3280A study. *J Clin Oncol*. 2013;31(suppl). Abstract 3001.
61. Mühlbauer M, Fleck M, Schütz C, et al. PD-L1 is induced in hepatocytes by viral infection and by interferon- $\alpha$  and - $\gamma$  and mediates T cell apoptosis. *J Hepatol*. 2006;45(4):520-528.
62. Freidin MB, Bhudia N, Lim E, et al. Impact of collection and storage of lung tumor tissue on whole genome expression profiling. *J Mol Diagn*. 2012;14(2):140-148.
63. Khleif S. MEDI4736, an anti-PD-L1 antibody with modified Fc domain: preclinical evaluation and early clinical results from a phase 1 study in patients with advanced solid tumors. In: Proceedings from the European Cancer Congress 2013; September 27-October 1, 2013; Amsterdam, The Netherlands. Abstract 802.
64. Mkrtychyan M, Najjar YG, Raulfs EC, et al. B7-DC-Ig enhances vaccine effect by a novel mechanism dependent on PD-1 expression level on T cell subsets. *J Immunol*. 2012;189(5):2338-2347.
65. Smothers F, Hoos A, Langermann S, et al. AMP-224, a fusion protein that targets PD-1. *Ann Oncol*. 2013;24(suppl 1):i7.
66. Rizvi NA, Infante JR, Gibney GT, et al. A phase I study of lirilumab (BMS-986015), an anti-KIR monoclonal antibody, administered with ipilimumab, an anti-CTLA-4 monoclonal antibody, in patients with select advanced solid tumors. *J Clin Oncol*. 2013;31(suppl):TPS3106. Abstract.
67. Jacobs R, Hintzen G, Kemper A, et al. CD56bright cells differ in their KIR repertoire and cytotoxic features from CD56dim NK cells. *Eur J Immunol*. 2001;31(10):3121-3126.
68. Carrega P, Morandi B, Costa R, et al. Natural killer cells infiltrating human nonsmall-cell lung cancer are enriched in CD56 bright CD16(-) cells and display an impaired capability to kill tumor cells. *Cancer*. 2008;112(4):863-875.
69. Björklund AT, Schaffer M, Fauriat C, et al. NK cells expressing inhibitory KIR for non-self-ligands remain tolerant in HLA-matched sibling stem cell transplantation. *Blood*. 2010;115(13):2686-2694.
70. Al Omar S, Middleton D, Marshall E, et al. Associations between genes for killer immunoglobulin-like receptors and their ligands in patients with solid tumors. *Hum Immunol*. 2010;71(10):976-981.
71. Al Omar SY, Marshall E, Middleton D, et al. Increased numbers but functional defects of CD56+ CD3+ cells in lung cancer. *Int Immunol*. 2012;24(7):409-415.
72. Al Omar SY, Marshall E, Middleton D, et al. Increased killer immunoglobulin-like receptor expression and functional defects in natural killer cells in lung cancer. *Immunology*. 2011;133(1):94-104.
73. Dorothée G, Echchakir H, Chansac BLM, et al. Functional and molecular characterization of a KIR3DL2/p140 expressing tumor-specific cytotoxic T lymphocyte clone infiltrating a human lung carcinoma. *Oncogene*. 2003;22(46):7192-7198.
74. Wiśniewski A, Jankowska R, Passowicz-Muszyńska E, et al. KIR2DL2/S2 and HLA-C C1C1 genotype is associated with better response to treatment and prolonged survival of patients with non-small cell lung cancer in a Polish Caucasian population. *Hum Immunol*. 2012;73(9):927-931.
75. Sanborn R. A phase I dose-escalation and cohort expansion study of lirilumab (anti-KIR; BMS-986015) administered in combination with nivolumab (anti-PD-1; BMS-936558; ONO-4538) in patients (Pts) with advanced refractory solid tumors. *J Clin Oncol*. 2013;31(suppl):TPS3110. Abstract.
76. Szoln M, Hodi F, Margolin K, et al. Phase I study of BMS-663513, a fully human anti-CD137 agonist monoclonal antibody, in patients (pts) with advanced cancer (CA). *J Clin Oncol*. 2008;26(15S):3007.
77. Matsuzaki J, Gnjatich J, Mhawech-Fauceglia P, et al. Tumor-infiltrating NY-ESO-1-specific CD8+ T cells are negatively regulated by LAG-3 and PD-1 in human ovarian cancer. *Proc Natl Acad Sci U S A*. 2010;107(17):7875-7880.
78. Okazaki T, Okazaki IM, Wang J, et al. PD-1 and LAG-3 inhibitory co-receptors act synergistically to prevent autoimmunity in mice. *J Exp Med*. 2011;208(2):395-407.
79. Woo SR, Turnis ME, Goldberg MV, et al. Immune inhibitory molecules LAG-3 and PD-1 synergistically regulate T-cell function to promote tumoral immune escape. *Cancer Res*. 2012;72(4):917-927.
80. Yin Y, Huang X, Lynn KD, et al. Phosphatidylserine-targeting antibody induces M1 macrophage polarization and promotes myeloid-derived suppressor cell differentiation. *Cancer Immunol Res*. 2013;1(4):256-268.
81. Gerber DE, Stopeck AT, Wong L, et al. Phase I safety and pharmacokinetic study of bavituximab, a chimeric phosphatidylserine-targeting monoclonal antibody, in patients with advanced solid tumors. *Clin Cancer Res*. 2011;17(21):6888-6896.
82. Digumarti R, Suresh A, Bhattacharyya G, et al. Phase II study of bavituximab plus paclitaxel and carboplatin in untreated locally advanced or metastatic non-small cell lung cancer: interim results. *J Clin Oncol*. 2010;28(15 suppl):7589. Abstract.
83. Dragnev K, Attili S, Gagua R, et al. A randomized, open-label, phase 2 trial of paclitaxel/carboplatin with or without bavituximab in patients with previously untreated locally advanced or metastatic non-squamous non-small-cell lung cancer. Paper presented at: Multidisciplinary Symposium in Thoracic Oncology; September 6, 2012; Chicago, IL.
84. Stewart RA, Mulgrew K, Wang S, et al. Blockade of PD-L1 mediated immunosuppression for cancer therapy-MEDI4736, monoclonal antibody discovery and preclinical development. Paper presented at: the 27th Annual Meeting of the Society for Immunotherapy of Cancer; October 26-28, 2012; North Bethesda, MD.
85. Armand P, Nagler A, Weller EA, et al. Disabling immune tolerance by programmed death-1 blockade with pidilizumab after autologous hematopoietic stem-cell transplantation for diffuse large B-cell lymphoma: results of an international phase II trial. *J Clin Oncol*. 2013 Oct 14. Epub ahead of print.
86. Weinberg AD, Thalhoffer C, Morris N, et al. Anti-OX40 (CD134) administration to nonhuman primates: immunostimulatory effects and toxicokinetic study. *J Immunother*. 2006;29(6):575-585.
87. van Cruisen H, Ruiz MG, van der Valk P, et al. Tissue micro array analysis of ganglioside N-glycolyl GM3 expression and signal transducer and activator of transcription (STAT)-3 activation in relation to dendritic cell infiltration and microvessel density in non-small cell lung cancer. *BMC Cancer*. 2009;9(1):180.
88. Blanco R, Rengifo CE, Cedeño M, et al. Immunoreactivity of the 14F7 Mab (Raised against N-Glycolyl GM3 Ganglioside) as a positive prognostic factor in non-small-cell lung cancer. *Patholog Res Int*. 2012;2012:235418.
89. de Leon J, Fernández A, Mesa C, et al. Role of tumour-associated N-glycosylated variant of GM3 ganglioside in cancer progression: effect over CD4 expression on T cells. *Cancer Immunol Immunother*. 2006;55(4):443-450.
90. Gomez DE, Vázquez AM, Alonso DF, et al. Anti-idiotypic antibodies in cancer treatment. *Front Oncol*. 2012;3:37.
91. Shaw AT, Yeap BY, Solomon BJ, et al. Effect of crizotinib on overall survival in patients with advanced non-small-cell lung cancer harbouring ALK gene rearrangement: a retrospective analysis. *Lancet Oncol*. 2011;12(11):1004-1012.
92. Paez JG, Jänne PA, Lee JC, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science*. 2004;304(5676):1497-1500.
93. Sloan JA, Cella D, Frost MH, et al. Assessing clinical significance in measuring oncology patient quality of life: introduction to the symposium, content overview, and definition of terms. *Mayo Clin Proc*. 2002;77(4):367-370.
94. Mina LA, Sledge GW Jr. Rethinking the metastatic cascade as a therapeutic target. *Nat Rev Clin Oncol*. 2011;8(6):325-332.
95. Sledge GW Jr. The challenge and promise of the genomic era. *J Clin Oncol*. 2012;30(2):203-209.
96. Ocaña A, Amir E, Vera F, et al. Addition of bevacizumab to chemotherapy for treatment of solid tumors: similar results but different conclusions. *J Clin Oncol*. 2011;29(3):254-256.
97. Niraula S, Seruga B, Ocana A, et al. The price we pay for progress: a meta-analysis of harms of newly approved anticancer drugs. *J Clin Oncol*. 2012;30(24):3012-3019.
98. Sullivan R, Peppercorn J, Sikora K, et al. Delivering affordable cancer care in high-income countries. *Lancet Oncol*. 2011;12(10):933-980.
99. Ribas A, Hersey P, Middleton MR, et al. New challenges in endpoints for drug development in advanced melanoma. *Clin Cancer Res*. 2012;18(2):336-341.