New strategies in immunotherapy for lung cancer: beyond PD-1/PD-L1

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Abstract: Immunotherapy has significantly altered the treatment landscape for many cancers, including non-small cell lung cancer (NSCLC). Currently approved immuno-oncology agents for lung cancer are aimed at the reversal of immune checkpoints, programmed death protein-1 (PD-1) and programmed death ligand-1 (PD-L1). Although responses to checkpoint inhibitors are encouraging, and in some cases durable, these successes are not universal among all treated patients. In order to optimize our treatment approach utilizing immunotherapy, we must better understand the interaction between cancer and the immune system and evasion mechanisms. In this review, we will provide an overview of the immune system and cancer, and review novel therapies that promote tumor antigen release for immune system detection, activate the effector T-cell response, and reverse inhibitory antitumor signals.

Keywords: metabolites, myeloid cell factors, TNF receptor superfamily [TNFRSF], tumor microenvironment, tumor vaccine

Introduction
Lung cancer is the leading cause of cancer deaths in the United States (US) and worldwide.1,2 Nearly 60% of lung cancers are diagnosed with metastatic disease where 5-year survival rates are still lower than 5%.3 However, with improvements in smoking cessation, early detection, and treatment of lung cancer, mortality rates have steadily declined over the past two decades.2 One of the treatment advances likely impacting survival is immunotherapy.

In 2015, two PD-1 inhibitors, nivolumab and pembrolizumab were US Food and Drug Administration (FDA) approved for metastatic non-small cell lung cancer (NSCLC) patients who progressed following first-line platinum-based therapy.4-6 In 2016, the first programmed death ligand-1 (PD-L1) inhibitor, atezolizumab, was also approved for the same indication.7,8 Unfortunately, objective response rates (ORRs) in the second-line setting still remain between 14–20% and median overall survival (OS) is reported at 9–13 months.4,5,8,9 In the treatment-naïve setting, monotherapy with programmed death protein-1 (PD-1) inhibitors have demonstrated variable success. For patients with a PD-L1 expression ≥50%, pembrolizumab monotherapy is associated with a superior progression-free survival (PFS), ORR, and median OS when compared with platinum-doublet chemotherapy.10,11 The phase III, Keynote 042 trial, confirmed these findings in the PD-L1 ≥ 50% expressers but failed to show an OS benefit for PD-L1 expression of 1–49%.12 Contrary to pembrolizumab, nivolumab did not result in a similar benefit over chemotherapy when used as monotherapy for a PD-L1 expression ≥5%.13 Recent phase III studies have demonstrated favorable outcomes when immunotherapy is combined with platinum-doublet chemotherapy in the first-line. The phase III studies combining pembrolizumab with platinum-doublet chemotherapy in nonsquamous (Keynote 189) and squamous histologies (Keynote 407) demonstrate superior OS, PFS, and ORR over platinum-doublet chemotherapy.14,15 The OS benefit persisted for PD-L1 expression <1%. Atezolizumab is also being evaluated with platinum-doublet chemotherapy for squamous and nonsquamous histologies.16,17 The recently published IMPower 150 phase III trial also
demonstrated an OS, PFS, and ORR benefit of atezolizumab, carboplatin, paclitaxel, and bevacizumab versus carboplatin, paclitaxel and bevacizumab in nonsquamous NSCLC. The PFS benefit continued to favor the atezolizumab-containing arm even for PD-L1 < 1% in both the tumor and immune cells; it remains to be seen if there will be a similar OS benefit for this subpopulation of patients. Lastly, attempts to combine PD-1/PD-L1 inhibitors with CTLA-4 inhibitors showed encouraging tumor responses despite increased toxicity in the initial phase I studies. Checkmate 227, a multipart phase III study assessed the role of different combinations of nivolumab versus chemotherapy in the setting of variable PD-L1 expressions and tumor mutational burden (TMB) as measured by the FoundationOne next generation sequencing assay. Using a predefined threshold of 10 mutations per megabase (mu/Mb) as a high TMB, the combination of nivolumab and ipilimumab demonstrated improved PFS and ORR compared with platinum-doublet chemotherapy. This benefit persisted regardless of tumor histology and PD-L1 status. Despite these initial encouraging findings, more patients in the immunotherapy arm discontinued therapy due to treatment-related toxicities (17.4% versus 8.9%). We are awaiting OS data for this study along with the phase III MYSTIC study [ClinicalTrials.gov identifier: NCT02453282]; the latter of which compared a durvalumab (a PD-L1 inhibitor) and tremelimumab (a CTLA-4 inhibitor). A list of selected first and second-line checkpoint immunotherapy trials are summarized in Table 1. Currently, the US FDA has approved pembrolizumab for the use in treatment-naïve metastatic NSCLC with a PD-L1 expression ≥50% and in combination with platinum-doublet chemotherapy. This cycle relies on the recognition and processing of tumor-specific neoantigens by dendritic cells. The neoantigens are presented via the major histocompatibility (MHC) class I and II molecules on antigen presenting cells (APCs) and bind to the corresponding T-cell receptor (TCR). Naive T-cells can then proliferate into effector memory T-cells, effector T-cells, or exhausted effector T-cells. This response is balanced by the presence of regulatory T-cells (Tregs) that limit damage to nontumor self-antigens. These activated effector T-cells are subsequently recruited into the TME resulting in the targeted killing of tumor cells. The neoantigens released from destroyed tumor cells further renews and augments this cycle.

Cancer is able to alter this cycle to its advantage and promote its survival. Chen and Mellman proposed that there exists a cancer-immune set point that is based upon the balance of immune stimulatory and inhibitory factors, both of which are influenced by the tumor and host genetics, and environment. One example is the impact of smoking and NSCLC immunity; a retrospective study of 114 KRAS-mutated NSCLC patients found that PD-L1 expression...
Table 1. Select first and second-line checkpoint inhibitor trials.

| Trial                           | Histology | Drug                      | Median OS (mon) | Median PFS (mon) | ORR (%) | Tumor biomarker | Reference or NCT |
|---------------------------------|-----------|---------------------------|-----------------|------------------|---------|-----------------|------------------|
| **Second line**                 |           |                           |                 |                  |         |                 |                  |
| Checkmate 057 (phase III)       | Nonsquamous | Nivo versus docetaxel   | 12.2 versus 9.4 (HR 0.73, 95% CI, 0.59–0.89, p = 0.002) | 2.3 versus 4.2 (HR 0.92, 95% CI, 0.77–1.11, p = 0.39) | 19 versus 12 (p = 0.02) | PD-L1: No threshold | Borghaei and colleagues\(^6\) |
| Checkmate 017 (phase III)       | Squamous  | Nivo versus docetaxel    | 9.2 versus 6.0 (HR 0.59, 95% CI, 0.44–0.79, p < 0.001) | 3.5 versus 2.8 (HR 0.62, 95% CI, 0.47–0.81, p < 0.001) | 20 versus 9 (p = 0.008) | PD-L1: No threshold | Brahmer and colleagues\(^5\) |
| Keynote 010 (phase III)         | All       | Pembro (2 mg/kg, 10 mg/kg) versus Docetaxel | 10.4 versus 12.7 versus 8.5 (HR 0.71, 95% CI, 0.58–0.88, p = 0.008, HR 0.61, 95% CI, 0.49–0.75, p < 0.001) | 3.9 versus 4.0 versus 4.0 (HR 0.88, 95% CI, 0.74–1.05, p = 0.07, HR 0.79, 95% CI, 0.66–0.94, p = 0.004) | 18 versus 18 versus 9 (p = 0.0002) | PD-L1: ≥1% | Herbst and colleagues\(^9\) |
| OAK (phase III)                 | All       | Atezo versus docetaxel   | 13.8 versus 9.6 (HR 0.73, 95% CI, 0.62–0.87, p = 0.0003) | 2.8 versus 4.0 (HR 0.95, 95% CI, 0.82–1.10, p = 0.49) | 14 versus 15 [NS] | PD-L1: No threshold | Rittmeyer and colleagues\(^9\) |
| **First line (monotherapy)**    |           |                           |                 |                  |         |                 |                  |
| Keynote 024 (phase III)         | All       | Pembro versus PD          | 30 versus 14.2 (HR 0.63, 95% CI, 0.47–0.86, p = 0.002) | 10.3 versus 6.0 (HR 0.50, 95% CI, 0.37–0.68, p < 0.001) | 45.5 versus 29.8 (p = 0.0031) | PD-L1: ≥50% | Reck and colleagues\(^10\) Brahmer and colleagues\(^11\) |
| Keynote 042 (phase III)         | All       | Pembro versus PD          | PD-L1 ≥1%; 16.7 versus 12.1 (HR 0.81, 95% CI, 0.71–0.93, p = 0.0018) | PD-L1 ≥1%; 5.4 versus 6.5 (HR 1.07, 95% CI, 0.94–1.21) | PD-L1 ≥1%; 27.3 versus 26.5 PD-L1 ≥1–49%; 13.6 versus 12.1 (HR 0.92, 95% CI, 0.77–1.11) | PD-L1: ≥1% | Lopes and colleagues\(^12\) [NCT02220894] |
| Checkmate 026 (phase III)       | All       | Nivo versus PD            | 14.4 versus 13.2 (HR 1.02, 95% CI, 0.80–1.30) | 4.2 versus 5.9 (HR 1.15, 95% CI, 0.91–1.45, p = 0.25) | 26 versus 33 (HR 1.07, 95% CI, 0.86–1.30) | PD-L1: ≥1% [results for ≥5%] | Carbone and colleagues\(^13\) |
| **First line (combination)**    |           |                           |                 |                  |         |                 |                  |
| Keynote 024 G cohort (phase II) | Nonsquamous | Pembro + CP versus CP    | NR versus 21.1 (HR 0.56, 95% CI, 0.32–0.95, p = 0.0151) | 24 versus 9.3 (HR 0.53, 95% CI, 0.33–0.86, p = 0.0049) | 57 versus 30 (p = 0.0016) | PD-L1: No threshold | Langer and colleagues\(^21\) Gentzler and colleagues\(^22\) [NCT02039674] |

(Continued)
## Table 1. (Continued)

| Trial | Histology | Drug | Median OS (mon) | Median PFS (mon) | ORR (%) | Tumor biomarker |
|-------|-----------|------|----------------|------------------|---------|----------------|
| Keynote 189 (phase III) | Nonsquamous | Pembrolizumab + CP versus CP | 15.9 | 6.4 | 0.8-1.8 | HR 0.49, CI 0.38-0.64, p < 0.001 |
| Keynote 227 (phase III) | Squamous | Pembrolizumab + CP versus CP | 15.1 | 6.4 | 0.8-1.8 | HR 0.49, CI 0.38-0.64, p < 0.001 |
| Checkmate 227 (phase III) | All | Pembrolizumab + CP versus CP | 19.5 | 11.7 | 1.7-11.1 | HR 0.49, CI 0.38-0.64, p < 0.001 |
| IMpower 150 (phase III) | Nonsquamous | Pembrolizumab + CP versus CP | 15.9 | 6.4 | 0.8-1.8 | HR 0.49, CI 0.38-0.64, p < 0.001 |
| MYSTIC | All | Durvalumab + tremelimub versus durvalumab versus PD | 15.1 | 6.4 | 0.8-1.8 | HR 0.49, CI 0.38-0.64, p < 0.001 |

**Note:** HR, hazard ratio; CI, confidence interval; NCT, ClinicalTrials.gov; NS, not significant; ORR, overall response rate; OS, overall survival; P, pemetrexed; Pac, paclitaxel; PD, platinum doublet; PD-L1, programmed death ligand 1; Pembrolizumab, PFS, progression-free survival; T, pembrolizumab; TMB, tumor mutational burden.
was reported in 64% of current or former smokers as compared with 13% of never-smokers. In addition, the strength of PD-L1 expression by immunohistochemistry (IHC) 2+/3+ versus 0/1+ was also associated with a higher smoking pack-year history. The explanation for a high PD-L1 expression may have to do with the increased mutational burden as seen in smokers versus nonsmokers. Therefore, targeting the hyperactive PD-1/PD-L1 axis would promote tumor specific T cell responses and help to rebalance the cancer-immune set point.

Currently, the most clinically recognized immunotherapy agents target PD-1/PDL-1 and CTLA-4. CTLA-4 is found on T-cells and is important in the initial T-cell activation by competing with the costimulatory receptor CD28 on APCs through the binding of CD80 (B7.1) or CD86 (B7.2). In this manner, CTLA-4 is important in preventing the overactivation of the immune system. In contrast, PD-L1 is found on tumor-infiltrating T-cells along with tumor cells, in the TME. Typically, PD-L1 binds to its receptor, PD-1, found on numerous cells including effector T-cells, Tregs, B-cells, and natural killer (NK) cells. The binding of PD-L1 to PD-1 acts to limit excessive immune activity in situations such as with chronic viral infections and cancer. Both PD-1/PD-L1 and CTLA-4 mechanisms are effective mechanisms by which the cancer can halt the cancer–immunity cycle.

Unfortunately, PD-L1 expression can vary depending on the cancer type and may have treatment implications with immunotherapy. Teng and colleagues proposed that tumors could be divided into one of four types based upon the presence or absence of TILs and the PD-L1 status. By using this model, we can construct a framework whereby immunotherapy can be most effective. In general NSCLC is thought to be immunogenic and anti-PD-1/PD-L1 antibodies would be most effective in the presence of TILs and high PD-L1 expression. One of the potential phenotypes is the absence of TILs in the setting of PD-L1 positivity. In this scenario, treatment with anti-PD-1/PD-L1 antibodies would be less effective, and would require a different approach to treatment such as by inducing immunogenic cell death to recruit TILs to the TME. In a different scenario, TILs can be present without PD-L1 expression. This suggests that alternative immunosuppressive mechanisms may be involved. Outside the PD-1/PD-L1 axis there exists other immune regulators such as myeloid-derived suppressor cells (MDSCs), tumor-associated macrophages (TAMs), NK cells, dendritic cells, B-cells, and various chemokines/cytokines which likely play large roles in the TME. Overall these factors underlie the complexity of the immune system outside of PD-1/PD-L1 alone.

These models provide a valuable framework to understand and develop treatment strategies. By using the cancer–immunity cycle, we can break down the current areas of clinical investigation into three parts: the release of cancer antigens, activation of the T-cell response, and regulation of the inhibitory immune response.

Promoting release of cancer antigens

Tumor vaccines

As demonstrated in the cancer–immunity cycle, a tumor-directed immune response first relies on the presence of tumor-specific antigens. Tumor vaccines bridge this gap by introducing tumor antigens to prime the host immune system to produce antigen-specific effector and memory T-cell responses. The currently studied vaccines can be divided broadly into those that target specific tumor antigens (antigen-specific) or numerous tumor cells (whole-cell). The aim of whole-cell vaccines is to broaden the exposure of tumor-associated antigens.

Results from completed phase III studies of vaccines targeting three different antigens have largely come with disappointing results. The melanoma-associated antigen-A3 (MAGE-A3) is expressed on cancer cells and found in normal testis and placental cells. Despite being found in normal adult tissue, its presence is not immunogenic because it does not harness human leukocyte antigen (HLA) molecules. MAGE-A3 is overexpressed in nearly 40% of stage I–II NSCLCs. The vaccine contains a recombinant form of MAGE-A3 with fusion protein D of *Haemophilus influenzae* along with an immune stimulant, AS02B. In a phase II study, patients with resected, MAGE-A3 positive, stage IB–II NSCLC, were randomized to adjuvant MAGE-A3 vaccinations or placebo. Despite all patients who tested for immunogenicity having developed anti-MAGE-A3 antibodies, there was
no benefit with a disease-free interval, disease-free survival (DFS) or OS. The subsequent phase III study (MAGRIT) included 2312 patients with resected phase IB–IIIA MAGE-A3-positive NSCLC; this study allowed adjuvant chemotherapy. Similar to the phase II trial, there was no benefit of the MAGE-A3 vaccine over placebo among the primary endpoint of DFS in the overall population and in those who did not receive adjuvant chemotherapy; additionally there was no OS benefit. Overall the treatment with the MAGE-A3 vaccine was well tolerated in both studies. Currently, a different MAGE-A3 vaccine is being studied in combination with pembrolizumab [ClinicalTrials.gov identifier: NCT02879760].

The mucin 1 (MUC-1) glycoprotein is another antigen found in NSCLC that pathologically promotes tumor cell growth via its interaction with cell surface receptors. MUC-1 has been targeted in the development of two vaccines, tecemotide (L-BLP25) and TG4010 (MVA-MUC1-IL2). Tecemotide consists of a synthetic lipopeptide that proved to be well tolerated and immunogenic in the phase I study. The phase III randomized clinical trial (START) compared tecemotide with placebo as a maintenance therapy in unresectable stage III NSCLC following chemoradiation. There was no OS benefit seen except for a subgroup that received concurrent chemoradiation. Although this subgroup analysis sparked a subsequent trial in patients receiving concurrent chemoradiation, the sponsor ultimately terminated the trial. This decision was made as a result of the negative findings of the phase I/II Japanese study in unresectable stage III NSCLC patients, the majority of whom received concurrent chemoradiation. There are no further studies with tecemotide at this time.

TG4010 is a constructed from the Modified Vaccinia Ankara that expresses both MUC-1 and interleukin (IL)-2. The phase IIb/III randomized controlled trial (TIME) compared chemotherapy in combination with TG4010 with placebo for treatment-naïve metastatic NSCLC. Their primary endpoint of median PFS was met; but there was only a 0.8 month absolute benefit [5.9 months versus 5.1 months, hazard ratio (HR) 0.74, 95% confidence interval (CI) 0.55–0.98, p = 0.019]. In addition, the study supported the use of a new biomarker, triple-positive activated lymphocytes (TrPAL), which is defined by the presence of low values of CD16, CD56, and CD69. These markers represent activated NK cells with the lowest values of TrPAL corresponding to the best response when combined with chemotherapy. In the subgroup of patients with less than or equal to the third quartile (Q3) of TrPAL, there was a significant benefit of PFS (HR 0.59, 95% CI 0.40–0.87) and OS (HR 0.59, 95% CI 0.40–0.87) over placebo. Furthermore, the addition of TG4010 was well tolerated and is currently being explored with nivolumab in advanced NSCLC [ClinicalTrials.gov identifier: NCT02823990].

Lastly, belagenpumatucel-L, is a type of whole-cell vaccine consisting of four NSCLC cell lines with a transforming growth factor β (TGF-β) antisense gene modification. This alteration improves the immunogenicity of the cancer cells to prime the host immune system. The phase III randomized controlled trial (STOP) compared belagenpumatucel-L with placebo as a maintenance therapy for stage IIIA–IV NSCLC patients who had no disease progression following platinum-based chemotherapy. The primary endpoint of OS was not met, however a prespecified analysis showed that patients who received chemoradiation prior to randomization demonstrated improved OS. This observation suggests the possible role of radiation therapy in the priming of the antitumor immune response. Currently other whole-cell vaccines, tergenpumatucel-L and viagenpumatucel-L (HS-110) are being studied in combination with immune checkpoint inhibitors [ClinicalTrials.gov identifiers: NCT02460367 and NCT02439450].

One of the perceived limitations to tumor vaccine monotherapy are the inhibitory signals of the TME, such as with Tregs, MDSCs, and other immune checkpoints. Given the limited responses of current tumor vaccine therapies, the current strategy focuses on enhancing the antitumor response by combining it with other immune therapies. Preclinical studies provide evidence for combining PD-1/PD-L1 or CTLA-4 inhibitors to tumor vaccines. Binder and colleagues demonstrated that exhausted CD8+ T-cells in a murine tumor model could be rescued minimally by increasing tumor-specific antigens via Salmonella typhimurium A1-R. In contrast, by adding an anti-PD-L1 antibody in combination with antigen priming, there was an increase in antigen-specific T-cells and improved tumor rejection.
Duraiswamy and colleagues further established that combining a tumor vaccine (GVAX, a GM-CSF transduced whole-cell tumor) with anti-CTLA-4 and anti-PD-1/PD-L1 antibodies increased antigen-specific CD8+ T-cells and enhanced tumor rejection when compared with dual antibody therapy without GVAX.62

Adoptive cell therapy (ACT)
In contrast with tumor vaccines that prime the effector and memory T-cell response with tumor antigens, ACT is the process whereby host lymphocytes are collected and modified, and then returned back to the patient. This process allows these engineered T-cells to directly target specific tumor-associated antigens for destruction. These cells can be modified with chimeric antigen receptors (CARs), TCRs, or with an expansion of TILs.

Both CAR T-cells and TCRs are genetically engineered to target a specific tumor-associated antigen (TAA).63 Modified TCRs are high affinity receptors that target a specific MHC–peptide complex and are prone to formation of mixed TCR dimers with unknown specificities. Comparatively, CAR T-cells are MHC-independent and contain a single chain antibody with TAA specificity linked to an intracellular signaling domain. Following the success of CAR T-cell therapy in hematologic malignancies, researchers are trying to expand this role to solid tumors, including NSCLC.64 The limitations in developing CAR T-cells and TCR in NSCLC are the selection of the ideal TAA with little to no expression in normal tissue. Some of these antigens being studied in early phase clinical trials include mesothelin (MSLN), MUC-1, carcinoembryonic antigen (CEA), glypican-3 (GPC3), human epidermal growth factor receptor 2 (HER2), and receptor tyrosine kinase like orphan receptor (ROR1). One of the main challenges in the development of CAR T-cell and TCR therapy is to limit off-tumor adverse effects. For example, off-tumor effects of treatment with MSLN immunotoxin, SS1P, can lead to the dose-limiting toxicity of pleuritis.65 In developing CAR T-cells, safety switches such as with inducible caspase-9 gene or RNA electroporation, have been successful in addressing these issues.66–68 In addition, the next hurdle for the CAR T-cell and TCR is to overcome the immune-tolerant state that the tumor microenvironment poses. PD-1 can similarly be upregulated in the setting of CAR T-cells and has shown to be effective when genetically engineered to the CAR T-cell itself.69,70

The utilization of ACT of TILs have shown success in previously treated metastatic melanoma with ORRs of around 40–50% and a minority with durable responses.71,72 There have been attempts to expand this use to NSCLC. An earlier study evaluated the use of TILs as postoperative treatment for Stage II–III NSCLC.73 Tissue samples were obtained from surgically resected primary lung lesions followed by the isolation of lymphocytes and cancer cells; these cells were expanded in a medium containing recombinant IL-2. Patients were stratified by stage and either received a TILs containing regimen or standard of care. The TILs were infused on day 0 along with daily subcutaneous injections of recombinant IL-2 until the maximum tolerated dose was achieved. Median OS favored the TIL arm versus standard of care treatment (22.4 months versus 14.1 months). The OS benefit was primarily seen in the most advanced disease with stage IIIB NSCLC patients receiving TILs with radiotherapy versus chemoradiation (23.9 months versus 73 months, p < 0.01). Widespread use of this therapy has been tempered by the ability to sufficiently produce enough TILs, not to mention the time it can take (up to 6–8 weeks) for this process. The application of a rapid expansion protocols that involves the stimulation of isolated TILs in culture with anti-CD3 antibody, irradiated peripheral blood mononuclear feeder cells, and IL-2, have been effective in melanoma.74 Recently, preclinical creation and expansion of TILs from resected early stage primary lung cancers using a rapid expansion protocol were promising.75 Despite these tumors having a small volume, sufficient TILs were available with this protocol after 2 weeks. Further clinical trials are anticipated and determining how it fits into the new wave of immunotherapy will be exciting.

Activating the T-cell response

Tumor necrosis factor receptor superfamily
The tumor necrosis factor receptor (TNFR) superfamily is a group of highly conserved type 1 transmembrane glycoproteins with cysteine-rich domains that possess both coinhibitory and costimulatory responses.76 Herein, we will review five members of this class with stimulatory effects currently in clinical investigation [OX40, CD27, glucocorticoid-induced tumor necrosis factor

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OX-40. OX-40 (CD134 or TNFRSF4) is expressed on activated T-cells and binds to the OX-40 ligand on activated APCs. Activation of OX-40 results in expansion of both effector and tumor activity. There are numerous anti-antibodies, thus restoring dendritic cell and anti-inhibited with agonistic anti-OX-40 monoclonal using a murine model showed that Tregs were finding over 72 hours. Additional studies grade 1–2 with lymphopenia being a transient effect. In a phase I study demonstrating that the agent was safe and well tolerated in patients with advanced, heavily pretreated melanoma, colorectal cancer and renal cell carcinoma. The only dose-limiting toxicity was transient asymptomatic hyponatremia. Similar to preclinical studies, they observed increased active effector T-cells and decreased Tregs. Responses were modest with two patients with renal cell carcinoma (RCC) showing long-term responses beyond 2 years. There is an ongoing phase II study to assess the combination of nivolumab with varilumab in advanced solid tumors [ClinicalTrials.gov identifier: NCT02335918].

OX-40 is not only to be important with controlling infections but potentially beneficial in the antitumor response. In a phase I study of patients with refractory metastatic solid tumors, administration of a murine agonistic anti-human OX-40 monoclonal antibody resulted in an increased proliferation of CD8+ and CD4+ T-cells. The function of OX-40 was further confirmed in the evaluation of a patient with autosomal recessive mutation (R65C) resulting in the inability of OX-40L to bind to its receptor. This patient developed early onset Kaposi’s sarcoma, a human herpesvirus-8 (HHV-8) induced endothelial tumor that can be seen in states of dysfunctional or deficient CD4+ T-cells, like human immunodeficiency virus (HIV). The lack of functional OX-40 results in impaired CD4+ T-cell responses to antigen exposure, decreasing memory CD4+ T-cells and CD8+ T-cells.

GITR. GITR (TNFRSF18) is expressed on naive CD4+ and CD8+ T-cells in addition to B-cells and NK cells. It is also constitutively expressed on Tregs. When GITR binds to its ligand (GITRL) on APCs, this results in increased expression of CD8+ and CD4+FoxP3+ T-cells (non-Tregs). Despite no objective responses per Response Evaluation Criteria in Solid Tumors criteria, tumor shrinkage was seen in 12 of the 30 enrolled patients. Adverse events were mainly grade 1–2 with lymphopenia being a transient finding over 72 hours. Additional in vivo studies using a murine model showed that Tregs were inhibited with agonistic anti-OX-40 monoclonal antibodies, thus restoring dendritic cell and antitumor activity. There are numerous anti-OX-40 monoclonal antibodies and one fusion protein undergoing clinical investigation as a single agent and in combination with other immune therapies. The fusion protein links the Fc portion of an immunoglobulin to OX-40L and has shown to be more potent than the monoclonal antibody counterpart in vivo studies. We await the results of these agents to evaluate if these benefits translate to human studies.

CD27. CD27 (TNFRSF7) differs from other members of the TNFRSF in that it is constitutively expressed on both naïve and activated effector T-cells. Its ligand, CD70, is transiently expressed on activated dendritic cells, B-cells and T-cells. Their interaction results in CD8+ T-cell effector and memory differentiation, B-cell synthesis, and NK cell activity.
Table 2. Select immune activating agents in clinical trial.

| Class                      | Drug                      | Sponsor            | Regimen                               | Indication                   | NCT               | Phase/status       |
|----------------------------|---------------------------|--------------------|---------------------------------------|------------------------------|-------------------|--------------------|
| TNF receptor superfamily   | OX-40 INCAGN01949 [mAb]   | Incyte Corp       | Monotherapy                           | Advanced solid tumor         | NCT02923349      | Phase I/II recruiting |
|                            |                           |                    |                                       |                              |                   |                    |
|                            |                           |                    |                                       |                              |                   |                    |
|                            | GSK3174998 [mAb]          | Glaxo Smith Kline | Monotherapy, Combination with Pembro  | Advanced solid tumor         | NCT02528357      | Phase I recruiting  |
|                            |                           |                    |                                       |                              |                   |                    |
|                            | MEDI0562 [mAb]            | Med-Immune Inc.   | Monotherapy                           | Advanced solid tumors        | NCT02318394      | Phase I completed  |
|                            |                           |                    |                                       |                              |                   |                    |
|                            | MEDI6383 [fusion protein] |                    | Combination with Durva or Tremi       | Advanced solid tumors        | NCT02705482      | Phase I recruiting  |
|                            |                           |                    |                                       |                              |                   |                    |
|                            | MOXR0916 [mAb]            | Genentech          | Monotherapy and in combination with Durva | Advanced solid tumors        | NCT02221960      | Phase I active, not recruiting |
|                            |                           |                    |                                       |                              |                   |                    |
|                            | CD27 Varililumab [CDX-1127] [mAb] | Celldex Therapeutics | Combination with Nivo | Advanced solid tumors        | NCT02335918      | Phase I/II active, not recruiting |
|                            |                           |                    |                                       |                              |                   |                    |
|                            | GITR BMS-986156           | Bristol-Myers Squibb | Monotherapy, Combination with Nivo   | Advanced solid tumors        | NCT02598960      | Phase I/II active, not recruiting |
|                            |                           |                    |                                       |                              |                   |                    |
|                            | GWN323                    | Novartis           | Monotherapy and in combination with PDR 001 [anti-PD-1 mAb] | Advanced solid tumors, lymphoma | NCT02740270      | Phase I/II recruiting |
|                            |                           |                    |                                       |                              |                   |                    |
|                            | INCAGN01876 [mAb]         | Incyte             | Monotherapy                           | Advanced solid tumors        | NCT02697519      | Phase I/II recruiting |
|                            |                           |                    |                                       |                              |                   |                    |
|                            | MEDI1873 [fusion protein] | Med-Immune LLC     | Monotherapy                           | Advanced solid tumors        | NCT03126110      | Phase I/II recruiting |
|                            |                           |                    |                                       |                              |                   |                    |
|                            | OMP-336B11 [engineered human protein] | Onco-Med Pharma | Monotherapy                           | Advanced solid tumors        | NCT03295942      | Phase I Recruiting |
|                            |                           |                    |                                       |                              |                   |                    |
|                            | TRX518 [mAb]              | Leap Therapeutics, Inc. | Monotherapy                           | Advanced solid tumors        | NCT01239134      | Phase I Recruiting |

(Continued)
| Class | Drug | Sponsor | Regimen | Indication | NCT          | Phase/status  |
|-------|------|---------|---------|------------|--------------|---------------|
| 4-1BB | Urelumab [BMS-663513] (mAb) | Bristol-Myers Squibb | Combination with Nivo | Advanced solid tumors | NCT02534506 | Phase I recruiting |
|       |      |         |         | Combination with nivolumab | Advanced solid tumors, NHL | NCT02253992 | Phase I/I recruiting |
|       |      |         |         | Monotherapy | Advanced solid tumors, NHL | NCT01471210 | Phase I completed |
|       | Utomilumab [PF-05082566] (mAb) | Pfizer | Monotherapy | Advanced solid tumors | NCT01307267 | Phase I active, not recruiting |
|       |      |         |         | Combination with PF-05082566 [4-1BB/CD137] agonist | Advanced solid tumors | NCT02315066 | Phase I recruiting |
|       |      |         |         | Combination with avelumab [A], avelumab and PF-0418600 [OX40 agonist mAb] | Advanced solid tumors | NCT02954812 | Phase I/I Ib/II recruiting |
|       |      |         |         | Combination with MK-3475 [PD-1 inhibitor] | Advanced solid tumors | NCT02179918 | Phase I completed |
| CD40  | ADC-1013 (mAb) | Alligator Bioscience AB | Monotherapy intratumoral and IV | Advanced solid tumors | NCT02379741 | Phase I completed |
|       | APX005M (mAb) | Apexigen, Inc. | Combination with Nivo | NSCLC, melanoma | NCT03123783 | Phase I/I recruiting |
|       |      |         |         | Combination with anti-CSFR-1 mAb [FPA008], combination with FPA008 and Nivo | NSCLC, melanoma, RCC | NCT03502330 | Phase I not yet recruiting |
|       | JNJ-66457107 (mAb) | Janssen | Monotherapy | Advanced solid tumors | NCT02829099 | Phase I/I recruiting |
|       | R07009789 (mAb) | Hoffmann-La Roche | Combination with Atezo | Advanced solid tumors | NCT02304393 | Phase I recruiting |
|       | SEA-CD40 (mAb) | Seattle Genetics, Inc. | Combination with Pembrolizumab | Advanced solid tumors, HL, DLBCL, indolent lymphoma | NCT02376699 | Phase I recruiting |

Atezo, avelumab; DLBCL, diffuse large B-cell lymphoma; Durva, durvalumab; GITR, glucocorticoid-induced tumor necrosis factor; HL, Hodgkin’s lymphoma; Ipi, Ipilimumab; IV, intravenous; mAb, monoclonal antibody; NCT, ClinicalTrials.gov; NHL, non-Hodgkin’s lymphoma; Nivo, nivolumab; NSCLC, non-small cell lung cancer; PD-1, programmed death protein-1; Pembrolizumab; RCC, renal cell carcinoma; TNF, tumor necrosis factor.
TILs and reducing the negative effects from Tregs.\textsuperscript{97} Currently there are numerous anti-GITR antibodies in clinical trials as monotherapy and in combination with PD-1 and CTLA-4 antibodies.

\textbf{4-1BB.} Similar to OX-40 and GITR, 4-1BB (CD137 or TNFRSF\textsuperscript{9}) is a costimulatory receptor found on activated T-cells and myeloid cells that are important in maintaining memory T-cells and expansion of antigen-specific CD8\textsuperscript{+} T-cells.\textsuperscript{98,99} Agonistic effects of 4-1BB results in a novel T-cell population with direct cytotoxic activity \textit{via} granzyme, perforin and Fas ligand pathways.\textsuperscript{100} There are currently two agonistic anti-4-1BB monoclonal antibodies currently studied in clinical trials (utomilumab or PF-05082566; urelumab or BMS-663513). The initial monotherapy studies were complicated by severe potentially fatal hepatitis that resulted in the termination of some studies. These toxicities were thought to be dose-related and are currently being studied with lower doses in combination with other cancer therapies such as checkpoint inhibitors.\textsuperscript{101}

Similar to other costimulatory receptors discussed, the rationale behind combining these agents with immune checkpoint inhibitors is appealing; by releasing the immune-suppressing signals of the tumor microenvironment and augmenting the recruitment of cytotoxic effector T-cells, this would hope to synergize the desired effect. Both anti-CTLA-4 and anti-PD-1 monoclonal antibodies are being tested in combination with anti-4-1BB demonstrating preclinical success.\textsuperscript{101} The combination of anti-CTLA-4 and anti-4-1BB antibodies resulted in long-lasting tumor eradication via CD8\textsuperscript{+} T-cells when compared with either agent alone.\textsuperscript{102} In addition, as noted in clinical studies, anti-4-1BB resulted in severe autoimmune hepatotoxicity that was also confirmed in this study. Interestingly, the addition of anti-CTLA-4 antibodies in combination mitigated this autoimmune effect. Similar findings of superior tumor eradication in combination with anti-PD-1 antibodies were also demonstrated in a murine model of squamous cell carcinoma of the lung.\textsuperscript{103} Clinical studies with these two monoclonal antibodies are ongoing. It should also be noted that 4-1BB is also being studied to enhance the effector T-cell response with other therapies such as adoptive T-cell and vaccine models. When combined with adoptive cytotoxic T-cell transfer into a murine melanoma model, there was prolonged survival of intratumoral effector T-cells resulting in tumor eradication.\textsuperscript{104}

\textbf{CD40.} Lastly, CD40 and its ligand CD40L (CD154) are expressed on a broad range of different cell types including APCs, B-cells, platelets and even nonhematopoietic cells like endothelial cells and smooth muscle cells.\textsuperscript{105} Their interaction is important in priming of dendritic cells to activate cytotoxic CD8\textsuperscript{+} T-cells in the antitumor response.\textsuperscript{106–108} Currently, there are six agonistic anti-CD40 monoclonal antibodies currently in early phase studies for advanced solid tumors. The first among the group was CP-870,893; in the phase I study, the most common adverse effect was grade 1–2 cytokine release syndrome marked by fever, rigors, rash, nausea, vomiting and myalgias that occurred minutes to hours after infusion. In addition, there were dose-related and transient hematologic and liver toxicities. Of the 29 patients studied (5 of which had NSCLC), objective responses were seen in 14\%.\textsuperscript{109} Unfortunately, there are no active trials in advanced NSCLC for CP-870,893 at this time. One potential solution to reduce the dose-limiting immune toxicities is through direct tumor injection to stimulate systemic immune responses. This concept has been studied with ADC-1013; in preclinical murine models, there was successful generation of tumorspecific cytotoxic T-cell activity.\textsuperscript{110} The current clinical trial [ClinicalTrials.gov identifier: NCT02379741] is completed and pending results. Owing to the limitations with monotherapy and dose-limiting immune toxicities, other strategies are currently being explored such as combining these agents with chemotherapy or immune checkpoint inhibitors.\textsuperscript{105}

\section*{Reversing inhibitory signals}

\textbf{Immunoglobulin superfamily} The immunoglobulin superfamily (IgSF) is an ever-growing list of additional immune checkpoints that are being studied in clinical trials. We review here, the current members of the IgSF for which there are active clinical trials; these include lymphocyte-activation gene 3 (LAG-3), T-cell immunoglobulin mucin 3 (TIM-3), T-cell immunoglobulin and ITIM domain (TIGIT), killer immunoglobulin-like receptor (KIR), and V-domain immunoglobulin
suppressor of T-cell activation (VISTA). Table 3 lists the active trials in this category.

**LAG-3.** LAG-3 (CD223) is an important cell surface molecule that can be found on both Tregs and effector CD8+ T-cells. Tregs maintain immune tolerance by inhibiting effector T-cells. LAG-3 is a CD4+ T-cell homolog that binds to MHC class II molecules, facilitating their suppressive function. In the setting of effector T-cell activation, LAG-3 is upregulated. In *in vitro* studies of knockout mice (LAG-3−/−) showed diminished control of Treg expression. Furthermore, inhibition of LAG-3 was found to reverse the state of CD8+ T-cell immune tolerance.

In addition to its role on Tregs, LAG-3 is also found in low levels on effector T-cells. Upon their activation, LAG-3 expression on effector T-cells is increased, thus ensuring T-cell homeostasis. Inhibition by antibodies against LAG-3 resulted in the expansion and activity of effector T-cells *in vivo* and *in vitro*. In murine models, the recruitment of TILs by antitumor vaccines in combination with LAG-3 antibodies further augmented the cytotoxic tumor response.

In the setting of immune-tolerant environments such as in cancer, TILs can coexpress both LAG-3 and PD-1, which is thought to work synergistically. Murine knockout models for both LAG-3 and PD-1 (LAG-3−/−;PDCD-1−/−) developed early, fatal multiorgan autoimmune disease as compared with mice containing only a single knockout gene. Furthermore, treatment with dual antibodies against both LAG-3 and PD-1 resulted in significant tumor shrinkage due to the increased recruitment of tumor-specific CD8+ T-cells as compared with monotherapy. A similar synergistic effect was also seen with chronic viral infections; in a murine model of chronic lymphocytic choriomeningitis virus infection, dual blockade of LAG-3 and PD-1 increased antigen-specific CD8+ T-cells and decreased viral load compared with untreated controls. Preclinical studies have also demonstrated the presence of LAG-3 expression on the TILs of other cancers such as melanoma, hepatocellular carcinoma, gastric cancer, and NSCLC. The synergistic effects of LAG-3 and PD-1 are currently being explored in multiple clinical trials. Dual blockade with anti-LAG-3 antibodies and currently approved PD-1 inhibitors, is an exciting novel combination which may have less toxicity compared with CTLA-4 combinations given that LAG-3 and PD-1 expression are primarily limited to TILs.

Lastly, it should also be noted that soluble LAG3 (sLAG-3), in contrast with LAG-3, when bound to MHC class II, results in dendritic cell maturation and migration to lymph nodes. It was also shown to induce tumor-specific CD8+ T-cell responses. An injectable, recombinant soluble LAG-3Ig fusion protein has shown to be safe in early phase studies involving metastatic breast cancer and RCC. There are ongoing studies assessing the benefit of this recombinant soluble LAG-3Ig fusion protein in addition to monoclonal antibodies against LAG-3.

**TIM-3.** TIM-3 is a membrane protein found on T helper 1 cells (Th1), a subset of CD4+ T-cells which are important for cell-mediated immunity. It also found on CD8+ T-cells, Tregs, and cells of the innate immune system. TIM-3 binds to its ligand, galectin-9, a carbohydrate-binding protein that can be found on lymphocytes. When bound together, the combination acts as an inhibitory signal, regulating Th1 responses and induction of peripheral tolerance. In naïve immune states, galectin-9 is expressed in high levels within numerous tissues such as in lymph nodes and the spleen. In the setting of immune activation, galectin-9 mRNA expression is downregulated to allow for the expansion of Th1 cells and promoting the inflammatory response. This response is balanced by the induction of galectin-9 expression by the resultant production of inflammatory cytokines, interferon-gamma (INF-γ) and IL-1β. Of note, TIM-3 has other receptors HMGB1 and Ceacam-1, which are important in maintaining its inhibitory function and may have a role as future checkpoint targets.

Similar to LAG-3, TIM-3 expression correlates with chronic viral infections and cancer. In addition, when PD-1 is coexpressed with TIM-3 on TILs, this produces a state of T-cell exhaustion whereby there is a lack of inflammatory cytokines in response to antigen exposure. Dual inhibition with antibodies against PD-1 and TIM-3 has a synergistic effect at restoring antitumor immunity and causing tumor regression as compared with monotherapy alone.
Table 3. Selected immune checkpoint inhibitors in clinical trials.

| Class                          | Drug                        | Sponsor                        | Regimen                                      | Indication                                                                 | NCT              | Phase/Status         |
|-------------------------------|-----------------------------|--------------------------------|----------------------------------------------|-----------------------------------------------------------------------------|------------------|----------------------|
| **Immunoglobulin superfamily** |                             |                                |                                              |                                                                             |                  |                      |
| TIM3                          | LY3321367 [mAb]             | Eli Lilly and Company          | Monotherapy, combination with anti-PD-L1 mAb | Advanced solid tumors                                                      | NCT03099109      | Phase I recruiting   |
|                               |                             |                                | LY3300054                                    |                                                                             |                  |                      |
|                               | MBG563 [mAb]                | Novartis                       | Monotherapy, combination with anti-PD-1 mAb  | Advanced solid tumors                                                      | NCT02608268      | Phase I recruiting   |
|                               |                             |                                | PDR001                                       |                                                                             |                  |                      |
|                               | TSR-022 [mAb]               | Tesaro                         | Monotherapy, combination with anti-PD-L1 mAb | Advanced solid tumors                                                      | NCT02817633      | Phase I recruiting   |
|                               | BMS-986016 [mAb]            | Bristol Myers                  | Monotherapy and in combination with nivolumab| First/second-line NSCLC with PD on/after anti-PD1/PDL1                     | NCT01968109      | Phase I/II recruiting|
|                               | LAG525 [mAb]                | Novartis                       | Monotherapy, combination with anti-PD-1 mAb  | Advanced solid tumors                                                      | NCT02460224      | Phase I/II recruiting|
|                               | MGD013 [mAb]                | Macro-genics                   | Monotherapy                                  | Advanced solid tumors, hematologic neoplasms                               | NCT03219268      | Phase I recruiting   |
|                               | REGN3767 [mAb]              | Regeneron Pharma               | Monotherapy, combination with anti-PD-1 mAb  | Advanced solid tumors                                                      | NCT03250832      | Phase I recruiting   |
|                               |                             |                                | REGN28101                                     |                                                                             |                  |                      |
|                               | TSR-033 [mAb]               | Tesaro                         | Monotherapy, combination with anti-PD-1 mAb  | Advanced solid tumors                                                      | NCT03538028      | Phase I recruiting   |
|                               | INCAGN022385 [mAb]          | Incyte Corp                    | Monotherapy                                  | Advanced solid tumors                                                      | NCT03119428      | Phase I recruiting   |
| TIGIT                         | OMP-313M32 [mAb]            | OncoMed Pharma                 | Monotherapy                                  | Advanced solid tumors                                                      | NCT01750580      | Phase I completed    |
|                               |                             |                                |                                              |                                                                             | NCT01714739      | Phase I active, not  |
|                               |                             |                                |                                              |                                                                             |                  | recruiting           |
| VISTA                         | CA-170 [SM]                 | Curis, Inc                     | Monotherapy                                  | Advanced solid tumors, lymphoma                                            | NCT02812875      | Phase I recruiting   |
| **Metabolites and myeloid cell factors** |                             |                                |                                              |                                                                             |                  |                      |
| IDO                           | Epacadostat (INCB24360) [IDOi] | Incyte Corp                    | Epacadostat combination with azacitidine and Pembro | Advanced solid tumors                                                      | NCT02959437      | Phase I/II active, not recruiting |
|                               |                             |                                | Epacadostat combination with anti-PD-1 mAb and chemotherapy |                                                                             | NCT03085914      | Phase I/II recruiting |
|                               |                             |                                | Epacadostat combination with Pembro          |                                                                             | NCT02178722      | Phase I/II active, not recruiting |
|                               |                             |                                | Epacadostat combination with Nivo and PD versus PD versus Nivo and PD | First line stage IV or recurrent NSCLC                                  | NCT03348904      | Phase III active not recruiting |

(Continued)
| Drug               | Class       | Regimen                                                                 | Sponsor                          | Indication                                                                 | NCT     | Phase/status          |
|--------------------|-------------|--------------------------------------------------------------------------|----------------------------------|----------------------------------------------------------------------------|---------|-----------------------|
| Epacadostat        | IDOi        | Combination with Pembrol versus Pembrol                                   | NewLink Genetics Corp            | First line stage IV NSCLC with PD-L1 ≥50%                                   | NCT03322566 | Phase II recruiting   |
|                    |             | Combination with Pembrol and PD chemotherapy versus Pembrol              | Corvus Pharma, Inc.              | First line stage IV NSCLC                                                   | NCT03347123 | Phase II recruiting   |
|                    |             | Combination with Pembrol and PD chemotherapy versus Pembrol              | Paclitaxel, Inc.                 | First line stage IV NSCLC                                                   | NCT03260387 | Phase II recruiting   |
|                    |             | Combination with Pembrol and PD chemotherapy versus Pembrol              | Paclitaxel, Inc.                 | First line stage IV NSCLC                                                   | NCT03265582 | Phase II recruiting   |
|                    |             | Combination with Pembrol and PD chemotherapy versus Pembrol              | Paclitaxel, Inc.                 | First line stage IV NSCLC                                                   | NCT03265582 | Phase II recruiting   |
|                    |             | Combination with Pembrol and PD chemotherapy versus Pembrol              | Paclitaxel, Inc.                 | First line stage IV NSCLC                                                   | NCT03265582 | Phase II recruiting   |
|                    |             | Combination with Pembrol and PD chemotherapy versus Pembrol              | Paclitaxel, Inc.                 | First line stage IV NSCLC                                                   | NCT03265582 | Phase II recruiting   |
|                    |             | Combination with Pembrol and PD chemotherapy versus Pembrol              | Paclitaxel, Inc.                 | First line stage IV NSCLC                                                   | NCT03265582 | Phase II recruiting   |
|                    |             | Combination with Pembrol and PD chemotherapy versus Pembrol              | Paclitaxel, Inc.                 | First line stage IV NSCLC                                                   | NCT03265582 | Phase II recruiting   |
|                    |             | Combination with Pembrol and PD chemotherapy versus Pembrol              | Paclitaxel, Inc.                 | First line stage IV NSCLC                                                   | NCT03265582 | Phase II recruiting   |

Table 3. (Continued)
is found in on both CD4+ and CD8+ TILs of many solid and hematologic malignancies, including NSCLC. Gao and colleagues evaluated 51 tissue specimens of patients with pathologically confirmed NSCLC and compared their TIM-3 expression with normal lung tissue. TIM-3 expression was found on the majority of Foxp3+ Tregs and were associated with more advanced disease. In addition, TIM-3 expression on NK cells was also shown to be associated with stage III/IV disease and a decreased OS in NSCLC. Based upon preclinical studies suggesting a synergistic effect with combination anti-PD-1 antibodies, anti-TIM-3 antibodies are currently being studied as monotherapy and in combination for NSCLC.

**TIGIT.** TIGIT is expressed on NK cells, effector/memory T-cells and Tregs. It has two ligands, CD155 (poliovirus receptor or PVR) and CD112, which are expressed on tumor cells, in addition to APCs and T-cells. TIGIT binds with a higher affinity to CD155 but must also compete with binding of CD155 to stimulatory (CD226) and inhibitory (CD96) signals. Binding of TIGIT to its receptor results in the inhibition of IL-12, shifting T-cell production away from the cell-mediated Th1, pathway, resulting in an immune-tolerant state. One hypothesis is that activation of the stimulatory signal, CD226, promotes cell-mediated killing that is balanced by the inhibitory signal, TIGIT, which competes for binding of CD155 with CD226. TIGIT remains an additional checkpoint important in the regulation of T-cell responses.

TIGIT is highly expressed in TILs within the tumor microenvironment. Along with PD-1, LAG-3, and TIM-3, the expression of TIGIT on CD8+ TILs resulted in a dysfunctional effector T-cell incapable for antitumor activity. The previous theme of synergistic activity with PD-1 inhibition in reversing the state of effector T-cell exhaustion also persists when combined with a TIGIT inhibitor. Interestingly there is evidence of synergistic activity with blockade of TIM-3 in the IgSF, thus potentially leading the way for future clinical trials combining the novel checkpoint inhibitors. Currently, there is one anti-TIGIT antibody in a phase I study for locally advanced and metastatic solid tumors. It remains to be seen if TIGIT will be combined with other immune checkpoint inhibitors or with alterations of its coinhibitory/stimulatory signals.

**KIR.** KIRs are a family of regulatory cell surface receptors that are responsible for maintaining the balance of NK-mediated cell killing against foreign cells and limiting autoimmune attack. KIRs are found on NK cells and CD8+ T-cells. NK cells are a part of the innate immune system that makes up roughly 10% of circulating human lymphocytes. Their role in protecting against infections and cancer is rooted in its ability to identify cells not expressing MHC class I molecules. In certain cancers such as in lung cancer, MHC class I molecules are downregulated to escape immune attack. The KIR and NKG2A/CD94 receptors are two NK cell inhibitory receptors that bind to HLA class I molecules. The downstream effects of KIRs vary depending on the extracellular domain. The presence of a long cytoplasmic tail (KIR2DL, KIR3DL) results NK cell inhibition while the short cytoplasmic tail (KIR3DS, KIR3DS) results in NK cell activation.

Tumor cells can also escape immune destruction by increasing the inhibitory subset of KIRs to escape cytotoxic T-cell killing. In NSCLC, NK cells were noted to be in decreased numbers and have reduced NK activation as compared with areas of the lung without cancer. The reduction in NK cells may also be related to the increase in Tregs found in the tumor microenvironment. Unfortunately, even NK cells that infiltrate the tumor have an impaired cytotoxic ability. Among the KIRs detected in NSCLC tumor cells or TILs, expressing KIR 2D (L1, L3, L4, S4) and KIR 3DL1 were poor prognostic indicators associated with decreased survival. It remains to be seen to what role KIRs are involved in this process.

Anti-KIR monoclonal antibodies, 1-7F9 and lirilumab (BMS-986015), are currently being studied in hematologic malignancies and solid tumors. In the preclinical study for 1-7F9, a humanized monoclonal antibody against KIR2DL1, KIR2DL2, and KIR2DL3 receptors, there was increased NK cell-mediated lysis of acute myeloid leukemia (AML) blasts. Lirilumab is the recombinant version with a stabilized hinge, which was also shown to be effective in lymphoma in combination with the anti-CD20 monoclonal antibody, rituximab. Currently lirilumab is being studied.
in combination with PD-1 and CTLA-4 inhibitors in advanced solid tumors.

Lastly, it should also be noted that the other NK cell checkpoint, NKG2A-CD94, has also shown to be a potential target in cancer cells.\(^{157}\) Anti-NKG2A-CD94 monoclonal antibody, monalizumab (IPH2201), is currently being combined with the anti-PD-L1 antibody, durvalumab, for advanced solid tumors. NKG2A-CD94 is a heterodimer that binds to a nonclassical HLA class I molecule, HLA-E.\(^{158}\) NKG2A-CD94 possesses similar regulatory properties on NK cells as with KIR and is dependent on binding to HLA-E. HLA-E has been noted in various hematologic malignancies and solid tumors, including NSCLC. However, high expressions of HLA-E in NSCLC were associated with a worsened OS.\(^{159}\) Studies demonstrate the lack of NK cell-mediated tumor activity within NSCLC; hopefully the monoclonal antibodies targeting the interactions of NKG2A-HLA-E and KIR-HLA class I molecules are able to reverse this process.

**VISTA.** VISTA is a member of the B7 family that shares similarities to immune checkpoints, PD-1 and CTLA-4.\(^{160}\) It is a very conserved protein that is found predominantly on hematopoietic cells in the myeloid lineage such as macrophages, dendritic cells, MDSCs, and neutrophils; they are also noted on Tregs.\(^{161}\) VISTA acts as both a ligand and receptor, and is noted to be upregulated within the TME. Preclinical studies have demonstrated that in murine models of solid tumors, overexpression of VISTA is associated with T-cell exhaustion and inhibition through monoclonal antibodies reverses this effect.\(^{162,163}\) It was also demonstrated that both PD-1 and VISTA are nonredundant checkpoint inhibitors that show synergistic activity with dual inhibition.\(^{164}\)

As a result, targeting VISTA in combination with other immune checkpoints is an area of interest. Unfortunately, the phase I study of a novel humanized IgG1 monoclonal antibody against VISTA (JNJ-61610588) was terminated by the pharmaceutical sponsor [ClinicalTrials.gov identifier: NCT02671955]. However, a novel agent, CA-170, is a small molecule inhibitor of PD-1, PD-L1/2, and VISTA-PD-1H that is currently undergoing phase I study. Initial results are promising without any dose-limiting toxicities and showing peripheral T-cell expansion.\(^{165}\)

**Metabolites and myeloid cell factors**

In addition to targeting additional immune checkpoint inhibitors of the TME, immunosuppressing metabolites have also become an area of interest. There are currently soluble inhibitors in trial for indoleamine 2,3-dioxygenase (IDO), adenosine, arginase, and other myeloid factors. Table 3 lists the active trials in this category.

**IDO.** IDO is an enzyme ubiquitously expressed on TILs and myeloid cells in various tissues sites including the lung.\(^{166}\) It functions in the catabolism of the amino acid tryptophan (Try), to kynurenine (Kyn) and its metabolites.\(^{167}\) Depletion of tryptophan results in an increase of a stress-response kinase called GCN2 that causes a decrease in T-cell activation.\(^{168}\) Furthermore, Kyn binds to the aryl hydrocarbon receptor, causing the production of Tregs.\(^{169}\)

NSCLC is among the solid tumors showing a high expression of IDO and was predominantly represented within the TME.\(^{166}\) IDO expression is mediated by a number of inflammatory signals including IFN-γ.\(^{170,171}\) Numerous studies attempt to indirectly measure IDO activity by calculating the ratio of Kyn to Try (Kyn/Try).\(^{167}\) A higher Kyn/Try ratio was seen not only in patients with NSCLC compared with healthy controls but also in more advanced versus early stage disease.\(^{172}\) In addition, in patients with stage III NSCLC who underwent induction chemotherapy followed by concurrent chemoradiation, elevated Kyn/Try ratios after induction chemotherapy were associated with decreased OS.\(^{173}\) These studies demonstrate that IDO expression plays an active role in maintaining the immune-tolerant environment in NSCLC and is a potential therapeutic target.

There have been two main IDO inhibitors (epacadostat and indoximod) evaluated in clinical studies and one IDO peptide vaccine.\(^{167}\) Epacadostat (INCBO24360) is a small molecule inhibitor of IDO1. Preclinical studies have shown in vitro and in vivo increases in T-cell, NK cell, and DC cell proliferation with reductions in Tregs, and suppression of tumor growth in murine models, respectively.\(^{174}\) In the phase I study, epacadostat was well tolerated with the most common side effects being fatigue, nausea, decreased appetite and vomiting; the recommended phase II dose achieved >90% IDO1 activity inhibition.\(^{175}\) Overall ORR was limited at 34.6% with the best response being stable.
disease. The lack of response may be due to need for combination therapy due to the presence of other immune checkpoints. Combination therapy trials with epacadostat are ongoing. Preliminary data on the phase I/II study combining epacadostat with pembrolizumab in the NSCLC cohort [ClinicalTrials.gov identifier: NCT02178722] showed a disease control rate of 57% (4/7) and 53% (9/17) for patients with a PD-L1 TPS \( \geq 50\% \) \textit{versus} \(<50\%\), respectively.\(^{177}\) Side effects were similar to the phase I study. Results from the phase III study of epacadostat in combination with pembrolizumab in unresectable advanced stage melanoma showed no PFS or OS benefit over pembrolizumab alone [ClinicalTrials.gov identifier: NCT02752074].\(^{178}\) We will await the results in NSCLC but should temper our expectations about this combination.

Indoximod (NLG-8189) is a second oral IDO inhibitor that has also shown to be well tolerated along with similar minimal responses as a single agent.\(^{179}\) Preclinical studies support tumor regression when indoximod is combined with cytotoxic chemotherapy, suggesting the importance of TILs in optimizing the tumor response to IDO inhibitors.\(^{180}\) The subsequent phase I study combining indoximod with docetaxel in advanced solid tumors (34% were NSCLC) was overall well tolerated and showed comparable disease control rates to epacadostat.\(^{181}\) Building upon these early data, there is an ongoing phase I/II study combining indoximod and docetaxel with tergenpumatucel-L [ClinicalTrials.gov identifier: NCT02460367]. Tergenpumatucel-L is a vaccine consisting of allogeneic lung cancer cells that express alpha-1,3-galactosyltransferase (\(\alpha[1,3]\)Gal), a carbohydrate for which we have established innate immune response against.\(^{182}\) In a phase II study with advanced NSCLC, 56% (9/16) of patients who progressed on the vaccine and subsequently received chemotherapy achieved a response.\(^{183}\) This study suggested that tergenpumatucel-L might sensitize patients to chemotherapy, hence the rationale of its addition to indoximod and docetaxel combination. It should also be noted that another IDO peptide vaccine from Denmark was shown to be well tolerated and effective for advanced NSCLC with remarkable long-term disease response.\(^{184}\) Overall, IDO remains a promising target that likely requires additional immune checkpoint inhibition or augmentation of TILs to the TME to maximize antitumor response.

Adenosine. Adenosine is a molecule that is produced as a byproduct of tumor cell killing and proinflammatory mediators such as hypoxia.\(^{185}\) Adenosine triphosphate (ATP) released is converted to adenosine monophosphate (AMP) by the enzyme CD39, and AMP is then converted to adenosine by the rate-limiting enzyme CD73. Adenosine then interacts with one of four G-protein coupled receptors (A1, A2A, A2B, A3). Adenosine receptors A2A and A2B have immune-suppressing properties, likely acting as a physiologic break to excess inflammation.

Tumor cells manipulate this process to promote an immune-tolerant environment.\(^{186}\) The ischemic tumor environment is associated with an upregulation of CD39, CD73, and Tregs to promote an adenosine-mediated immune-suppressing state.\(^{187,188}\) In many tumors including NSCLC, CD73 expression is a poor prognostic indicator.\(^{189}\) Preclinical studies show that tumors produce higher levels of adenosine than in normal tissue\(^{190}\) and reduce the activity of NK cells and effector T-cells.\(^{185}\) Inhibition of the A2A receptor was shown to decrease tumor cell growth in both \textit{in vitro} and \textit{in vivo} NSCLC models.\(^{191}\) Hence, CD73 and adenosine receptors (mainly A2A) have garnered interest as potential therapeutic targets.\(^{192}\) Anti-CD73 monoclonal antibodies are being tested as monotherapy and in combination with a PD-L1 inhibitor for advanced solid tumors [ClinicalTrials.gov identifiers: NCT02503774, NCT03549000, NCT03454451].\(^{193}\) There are at least three adenosine A2A receptor monoclonal antibodies in clinical trials [ClinicalTrials.gov identifiers: NCT02403193, NCT02655822, NCT03207867]. Like with other checkpoint inhibitors, a synergistic antitumor effect can be seen when combined with anti-CD73 and anti-A2A receptor blockade and is being evaluated in the current studies.\(^{193–195}\)

Lastly, in NSCLC, CD73 and A2A receptor expression was primarily seen in adenocarcinoma histology with epidermal growth factor receptor mutations, potentially warranting further investigation in combination with current targeted therapies for selected patients.\(^{189}\)

Arginase and other myeloid cell factors. L-Arginine is an important amino acid for the growth of T-cells and a pathway that is manipulated by tumor cells.\(^{12,196}\) Arginase-1 (ARG1) is an enzyme constitutively expressed on granulocytes and upregulated by MDSCs and TAMs. MDSCs are
derived from immature myeloid cells and promote an immunosuppressive state in the TME. One of the mechanisms by which MDSCs maintain this environment is through the depletion of L-arginine by upregulating ARG1, resulting in apoptosis of tumor-antigen specific effecter T-cells. Similar to depletion of Try, the depletion of extracellular L-arginine downregulates T-cell activity though the loss of expression of TCR CD3 zeta chain. Elevated ARG1 expression has been documented in NSCLC as compared with healthy controls. In vivo inhibition of ARG1 in a lung cancer murine model reduced tumor growth but was unsuccessful in mice that genetically lacked of functional T and B-cells. This finding highlights the importance of the need for TILs in addition to inhibition of ARG1. Currently, a single arginase inhibitor is being studied as monotherapy and in combination with nivolumab [ClinicalTrials.gov identifier: NCT02903914].

The TAMs are another subset of myeloid cells that can promote tumorigenesis in a similar fashion to MDSCs. They share features with M2 macrophages, which express high levels of IL-10, decreasing the production of effector T-cells and promoting tumor survival. The effects of TAMs include increased angiogenesis and metastases, tumor invasion, and resistance to apoptosis. TAMs also express PD-L1 and therefore an attractive target in combination with agents targeting tumorigenesis within the myeloid lineage. Chemokines (i.e. CCL2) and cytokines (i.e. CSF1R, IL-10, Tie2) involved in this dysfunctional process are under investigation. Agents targeting IL-10 and colony stimulating factor 1 receptor (CSF1R) are currently being studied in clinical trials.

IL-10 has the potential to express contrasting responses; at low levels it can produce an immunosuppressive response but at high concentrations, it can promote the proliferation of CD8+ T-cells. Reversal of its immunosuppressive response with an anti-IL-10 antibody resulted in increased IL-12 expression and cytotoxic T-cell activity in a breast cancer murine model. Alternatively, in a phase I study of patients with advanced solid tumors, pegylated recombinant IL-10 (AM0010) is being evaluated for its ability to stimulate the expansion of CD8+ TILs [ClinicalTrials.gov identifier: NCT02009449]. As a single agent, it was well tolerated but responses were limited; the experimental phase in combination with chemotherapy or immunotherapy is ongoing.

CSF1R binding to its ligand CSF1 is important to allow for continued immune-suppressing actions of TAMs and other myeloid cells. Inhibition of CSF1R not only reduces the amount of TAMs, but also increases CD8+ T-cells. Numerous CSF1R inhibitors (small molecules and monoclonal antibodies) are currently being studied in clinical trials as monotherapy and in combination with both chemotherapy and immunotherapy. Overall, these agents are well tolerated with common side effects including fatigue, transaminitis, and facial/peripheral edema. NSCLC has demonstrated increased TAMs as noted by an increase of M2 macrophages in all histologies except large cell carcinoma. In addition, high levels of IL-10 expression in TAMs were associated with more advanced NSCLC stage and poor histologic differentiation. Therefore, given the increase of TAMs in NSCLC, the potential role for CSF1R inhibitors is promising.

**Tumor biomarkers**

Immunotherapy in advanced NSCLC is being moved towards the front-line setting either as monotherapy or in combination with systemic chemotherapy. We must be cognizant of the fact that not all patients benefit equally from immunotherapy. Few patients have durable responses as noted by the plateau in the Kaplan–Meier PFS curves, but some patients have inferior responses when compared with chemotherapy as suggested by the initial crossing of PFS curves. How to best predict which patients will benefit the most from immunotherapy is an important question that remains unanswered. PD-L1 is the tumor biomarker most often used but it does have its limitations. PD-L1 expression does not always predict response to PD-1 and PD-L1 inhibitors. In phase III studies of previously treated advanced NSCLC, nivolumab and atezolizumab, even patients with PD-L1 <1% expression had OS and PFS benefit over docetaxel. Despite this, higher PD-L1 expression is generally associated with a comparatively greater response to immunotherapy. Moreover, the tumor heterogeneity of PD-L1 expression and availability of numerous PD-L1 assays used for the various immune checkpoint inhibitors caution...
Attempts at identifying additional tumor biomarkers have been explored. The heterogeneity of somatic mutations among various tumor types has been previously reported, with NSCLC demonstrating a range of mutations (0.1–100 somatic mutations per Mb) but is particularly highest in smokers versus never-smokers. It is proposed that a high TMB is associated with increased neo-antigen exposure to APCs, thereby activating the tumor immune response. Rizvi and colleagues evaluated TMB through whole exome sequencing to determine the amount of somatic nonsynonymous mutations in patients with NSCLC who were treated with pembrolizumab. In tumors with >178 nonsynonymous mutations per tumor, labeled as high TMB, there was an improved response to pembrolizumab as compared with tumors with low TMB. They also reported improved ORR and PFS in patients harboring a molecular smoking signature marked by C-to-A transversions. The utility of TMB was also explored in a nonprespecified analysis of the Checkmate 026 phase III study that compared nivolumab monotherapy with chemotherapy in treatment-naive advanced NSCLC patients. Whole exome sequencing was used to determine TMB, with >242 mutations per tumor being considered as high TMB. As this was an exploratory analysis, patient with tumors of high TMB were unbalanced (30% in the nivolumab arm, 39% in the chemotherapy arm). Interestingly, ORR in the high TMB group was numerically higher with nivolumab over chemotherapy (47% versus 28%) along with the PFS (9.7 months versus 5.8 months). There was no association between the level of PD-L1 and TMB status. Patients with both PD-L1 expression of >50% and high TMB were found to have an ORR of 75% versus 25% in patients treated with nivolumab (n = 16) versus chemotherapy (n = 32), respectively. The post-hoc nature of these results limits our adoption of TMB until more prospective studies are completed. Recently published results from the multipart phase III trial, Checkmate 227, met its coprimary endpoint of PFS among patients with high TMB (≥10 mu/Mb) in advanced, treatment-naïve NSCLC, regardless of PD-L1 status, who were treated with combination nivolumab and ipilimumab versus chemotherapy. Patients with high versus low TMB had significantly greater median and 12-month PFS (42.6% versus 13.2%, HR 0.58, 95% CI 0.41–0.81, p < 0.0001). Responses were also durable at 12-months (68% versus 25%). Among patients with high TMB, the 12-month PFS rate was similarly improved over chemotherapy among the PD-L1 ≥1% versus <1% (42% versus 16% and 45% versus 8%, respectively). The trial also included treatment with nivolumab monotherapy for patients with PD-L1 ≥ 1%; there was no improvement in PFS regardless of TMB status. Although OS data is immature, this study supports TMB as a useful biomarker outside of PD-L1 in identifying patients who may benefit from combination immunotherapy.

Lastly, Teff gene signature expression is another biomarker that has gained interest. It was introduced in the phase II POPLAR study comparing atezolizumab monotherapy to docetaxel for previously treated advanced NSCLC. It initially included the expression of eight genes (CD8A, GZMA, GZMB, IFNγ, EOMES, CXCL9, CXCL10, and TBX21) believed to be associated with pre-existing immunity and PD-L1 expression on immune cells. The Teff-high versus low cohort was based upon gene expression at or above the median level versus below the median level, respectively. The Teff gene signature was refined to include the expression of three messenger RNAs (PD-L1, CXCL9, and INF-γ) and applied to the subsequent phase III OAK trial that compared atezolizumab with docetaxel in previously treated advanced NSCLC patients. The biomarker was found to be a more sensitive indicator for PFS compared with PD-L1 expression. The recent phase III IMPower 150 trial evaluating first-line atezolizumab with carboplatin, paclitaxel, and bevacizumab to carboplatin, paclitaxel and bevacizumab included PFS benefit in the Teff-high population as one of its coprimary endpoints. The PFS was significantly longer in the atezolizumab-containing arm and this benefit persisted for the Teff-high (11.3 months versus 6.8 months, HR 0.51, 95% CI, 0.38–0.68, p < 0.0001) and Teff-low cohorts (7.3 months versus 7.0 months, HR 0.76, 95% CI, 0.60–0.96). Prolonged PFS was seen regardless of PD-L1 status; responses of the Teff-high and Teff-low were comparable with tumor cell (TC) or immune cell (IC) 1/2/3 (PD-L1 ≥ 1% of tumor cells or tumor-infiltrating immune cells) and TC or IC 0 (PD-L1 < 1% of tumor cells or...
tumor-infiltrating immune cells), respectively. Given the similar benefit of Teff-high and PD-L1 ≥ 1%, the exact utility of Teff in addition to PD-L1 warrants further investigation. Identifying a consistent and predictable biomarker will become crucial in the new era of immunotherapeutic combinations. Although PD-L1 expression continues to be the gold standard at this time, I suspect that a combination of tumor biomarkers will be needed in deciding the proper therapeutic approach.

**Conclusion**

The immune system plays an important role in not only eradicating disease but also promoting long-lasting immunity. As we better understand how the immune system responds to cancer, we can specifically target the mechanisms whereby TCs evade destruction beyond PD-1/PDL-1 and CTLA-4. We now have a surplus of clinical trials evaluating the role of new immune checkpoints, immune-suppressing cytokines and metabolites, and costimulatory signals. In addition to focusing on shifting the cancer-immune set point towards T-cell stimulation, we also understand the importance of TILs in the antitumor response. Tumor vaccines are now being studied in combination with immune checkpoint inhibitors to improve tumor-associated T-cell activity that appeared to be lacking with monotherapy. Building upon the success of ACT in hematologic malignancies, the development of CAR T-cells for solid tumors like NSCLC are promising, especially in tumors lacking appropriate TILs. Models of the interactions between the host immune system and cancer help to provide an excellent framework to understand this complex process and allow us to understand that we cannot adopt a ‘one size fits all’ approach when it comes to immunotherapy. We described therapies in development that target three main areas of active research: the release of cancer antigens, activation of the T-cell response, and regulation of the inhibitory immune response. Based on preclinical studies and ongoing clinical trials with novel agents, it appears that we must adopt a combination approach. However, determining the most efficacious combination (i.e. two immune checkpoint inhibitors, costimulatory and checkpoint inhibitors, tumor vaccine and immune checkpoint inhibitors) while minimizing immune-related and financial toxicities will be the next hurdles. In addition, understanding how to personalize this treatment as proposed by Teng and colleagues will also be a challenge. Despite these unanswered questions, there is no doubt that the era of immunotherapy has been met with great success and growing optimism. We must work to further understand of this complex system if we hope to build on the current early success of immunotherapy.

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**Conflict of interest statement**

NV has no conflicting interests. LB has participated in advisory boards for Takeda, AstraZeneca, Novartis, Genentech.

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**References**

1. Fitzmaurice C, Allen C, Barber RM, et al. Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life-years for 32 cancer groups, 1990 to 2015: a systematic analysis for the global burden of disease study. *JAMA Oncol* 2017; 3: 524–548.

2. Siegel RL, Miller KD and Jemal A. Cancer statistics, 2017. *CA Cancer J Clin* 2017; 67: 7–30.

3. Howlader NNA, Krapcho M, Miller D, et al. (eds). SEER cancer statistics review, 1975–2014. National Cancer Institute, April 2017.

4. Borghaei H, Paz-Ares L, Horn L, et al. Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. *N Engl J Med* 2015; 373: 1627–1639.

5. Brahmer J, Reckamp KL, Baas P, et al. Nivolumab versus docetaxel in advanced squamous-cell non-small-cell lung cancer. *N Engl J Med* 2015; 373: 123–135.

6. Garon EB, Rizvi NA, Hui R, et al. Pembrolizumab for the treatment of non-small-cell lung cancer. *N Engl J Med* 2015; 372: 2018–2028.

7. Fehrenbacher L, Spira A, Ballinger M, et al. Atezolizumab versus docetaxel for patients with
previously treated non-small-cell lung cancer (POPLAR): a multicentre, open-label, phase 2 randomised controlled trial. *Lancet* 2016; 387: 1837–1846.

8. Rittmeyer A, Barlesi F, Waterkamp D, et al. Atezolizumab versus docetaxel in patients with previously treated non-small-cell lung cancer (OAK): a phase 3, open-label, multicentre randomised controlled trial. *Lancet* 2017; 389: 255–265.

9. Herbst RS, Baas P, Kim DW, et al. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. *Lancet* 2016; 387: 1540–1550.

10. Reck M, Rodriguez-Abreu D, Robinson AG, et al. Pembrolizumab versus chemotherapy for PD-L1-positive non-small-cell lung cancer. *N Engl J Med* 2016; 375: 1823–1833.

11. Brahmer J, Rodriguez-Abreu D, Robinson A, et al. OA 17.06 Updated analysis of KEYNOTE-024: pembrolizumab vs platinum-based chemotherapy for advanced NSCLC with PD-L1 TPS ≥50%. *J Thorac Oncol* 2017; 12: S1793–S1794.

12. Lopes G, Wu Y-L, Kudaba I, et al. Pembrolizumab (pembro) versus platinum-based chemotherapy (chemo) as first-line therapy for advanced/metastatic NSCLC with a PD-L1 tumor proportion score (TPS) ≥ 1%: open-label, phase 3 KEYNOTE-042 study. *J Clin Oncol* 2018; 36(Suppl): abstract LBA4.

13. Carbone DP, Reck M, Paz-Ares L, et al. First-line nivolumab in stage IV or recurrent non-small-cell lung cancer. *N Engl J Med* 2017; 376: 2415–2426.

14. Gandhi L, Rodriguez-Abreu D, Gadgeel S, et al. Pembrolizumab plus chemotherapy in metastatic non-small-cell lung cancer. *N Engl J Med* 2018; 378: 2078–2092.

15. Paz-Ares LG, Luft A, Tafereshi A, et al. Phase 3 study of carboplatin-paclitaxel/nab-paclitaxel (Chemo) with or without pembrolizumab (Pembro) for patients (Pts) with metastatic squamous (Sq) non-small cell lung cancer (NSCLC). *J Clin Oncol* 2018; 36(Suppl.): abstract 105.

16. Jotte RM, Cappuzzo F, Vynnychenko I, et al. IMpower131: primary PFS and safety analysis of a randomized phase III study of atezolizumab + carboplatin + paclitaxel or nab-paclitaxel vs carboplatin + nab-paclitaxel as 1L therapy in advanced squamous NSCLC. *J Clin Oncol* 2018; 36(Suppl.): abstract LBA9000.

17. Socinski MA, Jotte RM, Cappuzzo F, et al. Atezolizumab for first-line treatment of metastatic nonsquamous NSCLC. *N Engl J Med* 2018; 378: 2288–2301.

18. Antonia S, Goldberg SB, Balmanoukian A, et al. Safety and antitumour activity of durvalumab plus tremelimumab in non-small cell lung cancer: a multicentre, phase 1b study. *Lancet Oncol* 2016; 17: 299–308.

19. Hellmann MD, Rizvi NA, Goldman JW, et al. Nivolumab plus ipilimumab as first-line treatment for advanced non-small-cell lung cancer (CheckMate 012): results of an open-label, phase 1, multicohort study. *Lancet Oncol* 2017; 18: 31–41.

20. Hellmann MD, Ciuleanu TE, Pluzanski A, et al. Nivolumab plus ipilimumab in lung cancer with a high tumor mutational burden. *N Engl J Med* 2018; 378: 2093–2104.

21. Langer CJ, Gadgeel SM, Borghaei H, et al. Carboplatin and pemetrexed with or without pembrolizumab for advanced, non-squamous non-small-cell lung cancer: a randomised, phase 2 cohort of the open-label KEYNOTE-021 study. *Lancet Oncol* 2016; 17: 1497–1508.

22. Gentzler RD, Langer CJ, Borghaei H, et al. 24-month overall survival from KEYNOTE-021 cohort G: pemetrexed-carboplatin plus pembrolizumab as first-line therapy for advanced nonsquamous NSCLC. *J Clin Oncol* 2018; 36(Suppl.): abstract 9026.

23. Reck M, Socinski MA, Cappuzzo F, et al. LBA1_PR: primary PFS and safety analyses of a randomized phase III study of carboplatin + paclitaxel +/− bevacizumab, with or without atezolizumab in 1L non-squamous metastatic NSCLC (IMPOWER150). *Ann Oncol* 2017; 28.

24. Horn L, Spigel DR, Vokes EE, et al. Nivolumab versus docetaxel in previously treated patients with advanced non-small-cell lung cancer: two-year outcomes from two randomized, open-label, phase III trials (checkmate 017 and checkmate 057). *J Clin Oncol* 2017; JCO2017743062.

25. Restifo NP, Smyth MJ and Snyder A. Acquired and intrinsic resistance in cancer immunotherapy. *Mol Oncol* 2014; 8: 1132–1139.
28. Sacher AG and Gandhi L. Biomarkers for the clinical use of PD-1/PD-L1 inhibitors in non-small-cell lung cancer: a review. *JAMA Oncol* 2016; 2: 1217–1222.

29. Chen DS and Mellman I. Oncology meets immunology: the cancer-immune cycle. *Immunity* 2013; 39: 1–10.

30. Chen DS and Mellman I. Elements of cancer immunity and the cancer-immune set point. *Nature* 2017; 541: 321–330.

31. Calles A, Liao X, Sholl LM, et al. Expression of PD-1 and its ligands, PD-L1 and PD-L2, in smokers and never smokers with KRAS-mutant lung cancer. *J Thorac Oncol* 2015; 10: 1726–1735.

32. Rizvi NA, Hellmann MD, Snyder A, et al. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science* 2015; 348: 124–128.

33. Govindan R, Ding L, Griffith M, et al. Genomic landscape of non-small cell lung cancer in smokers and never-smokers. *Cell* 2012; 150: 1121–1134.

34. Wing K, Onishi Y, Prieto-Martin P, et al. CTLA-4 control over Foxp3 + regulatory T-cell function. *Science* 2008; 322: 271–275.

35. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer* 2012; 12: 252–264.

36. Herbst RS, Soria JC, Kowanetz M, et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. *Nature* 2014; 515: 563–567.

37. Day CL, Kaufmann DE, Kiepiela P, et al. PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression. *Nature* 2006; 443: 350–354.

38. Gatalica Z, Snyder C, Maney T, et al. Programmed cell death 1 (PD-1) and its ligand (PD-L1) in common cancers and their correlation with molecular cancer type. *Cancer Epidemiol Biomarkers Prev* 2014; 23: 2965–2970.

39. Teng MW, Ngiow SF, Ribas A, et al. Classifying cancers based on T-cell infiltration and PD-L1. *Cancer Res* 2015; 75: 2139–2145.

40. Bremnes RM, Busund LT, Kleveland TL, et al. The role of tumor-infiltrating lymphocytes in development, progression, and prognosis of non-small cell lung cancer. *J Thorac Oncol* 2016; 11: 789–800.

41. Pauken KE and Wherry EJ. Overcoming T cell exhaustion in infection and cancer. *Trends Immunol* 2015; 36: 265–276.

42. Gabrilovich DI, Ostrand-Rosenberg S and Bronte V. Coordinated regulation of myeloid cells by tumours. *Nat Rev Immunol* 2012; 12: 253–268.

43. Caliguri MA. Human natural killer cells. *Blood* 2008; 112: 461–469.

44. Gajewski TF, Schreiber HD and Fu YX. Innate and adaptive immune cells in the tumor microenvironment. *Nat Immunol* 2013; 14: 1014–1022.

45. Cuppens K and Vansteenkiste J. Vaccination therapy for non-small-cell lung cancer. *Curr Opin Oncol* 2014; 26: 165–170.

46. Adam V, Wauters I and Vansteenkiste J. Melanoma-associated antigen-A3 vaccination in the treatment of non-small-cell lung cancer. *Expert Opin Biol Ther* 2014; 14: 365–376.

47. Sielen W, Varwerk C, Linder A, et al. Melanoma associated antigen (MAGE)-A3 expression in stages I and II non-small cell lung cancer: results of a multi-center study. *Eur J Cardiothorac Surg* 2004; 25: 131–134.

48. Vansteenkiste J, Zielinski M, Linder A, et al. Adjuvant MAGE-A3 immunotherapy in resected non-small-cell lung cancer: phase II randomized study results. *J Clin Oncol* 2013; 31: 2396–2403.

49. Vansteenkiste JF, Cho BC, Vanakesa T, et al. Efficacy of the MAGE-A3 cancer immunotherapeutic as adjuvant therapy in patients with resected MAGE-A3-positive non-small-cell lung cancer (MAGRIT): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Oncol* 2016; 17: 822–835.

50. Raina D, Kosugi M, Ahmad R, et al. Dependence on the MUC1-C oncoprotein in non-small cell lung cancer cells. *Mol Cancer Ther* 2011; 10: 806–816.

51. Butts C, Murray RN, Smith CJ, et al. A multicenter open-label study to assess the safety of a new formulation of BLP25 liposome vaccine in patients with unresectable stage III non-small-cell lung cancer. *Clin Lung Cancer* 2010; 11: 391–395.

52. Butts C, Socolinski MA, Mitchell PL, et al. Tecemotide (L-BLP25) versus placebo after chemoradiotherapy for stage III non-small-cell lung cancer (START): a randomised, double-blind, phase 3 trial. *Lancet Oncol* 2014; 15: 59–68.

53. Katakami N, Hida T, Nokihara H, et al. Phase I/II study of tecemotide as immunotherapy in Japanese patients with unresectable stage III non-small cell lung cancer. *Lung Cancer* 2017; 105: 23–30.
54. Limacher JM and Quoix E. TG4010: a therapeutic vaccine against MUC1 expressing tumors. *Oncoimmunology* 2012; 1: 791–792.

55. Quoix E, Lena H, Losonczy G, et al. TG4010 immunotherapy and first-line chemotherapy for advanced non-small-cell lung cancer (TIME): results from the phase 2b part of a randomised, double-blind, placebo-controlled, phase 2b/3 trial. *Lancet Oncol* 2016; 17: 212–223.

56. Quoix E, Ramlau R, Westeel V, et al. Therapeutic vaccination with TG4010 and first-line chemotherapy in advanced non-small-cell lung cancer: a controlled phase 2B trial. *Lancet Oncol* 2011; 12: 1125–1133.

57. Nemunaitis J, Dillman RO, Schwarzenberger PO, et al. Phase II study of belagenpumatucel-L, a transforming growth factor-beta-2 antisense gene-modified allogeneic tumor cell vaccine in non-small-cell lung cancer. *J Clin Oncol* 2006; 24: 4721–4730.

58. Tzai TS, Shiau AL, Liu LL, et al. Immunization with TGF-beta antisense oligonucleotide-modified autologous tumor vaccine enhances the antitumor immunity of MBT-2 tumor-bearing mice through upregulation of MHC class I and Fas expressions. *Anticancer Res* 2000; 20: 1557–1562.

59. Giaccone G, Bazhenova LA, Nemunaitis J, et al. A phase III study of belagenpumatucel-L, an allogeneic tumour cell vaccine, as maintenance therapy for non-small cell lung cancer. *J Clin Oncol* 2006; 24: 4721–4730.

60. Thomas A and Giaccone G. Why has active immunotherapy not worked in lung cancer? *Ann Oncol* 2015; 26: 2213–2220.

61. Binder DC, Engels B, Arina A, et al. Antigen-specific bacterial vaccine combined with anti-PD-L1 rescues dysfunctional endogenous T cells to reject long-established cancer. *Cancer Immunol Res* 2013; 1: 123–133.

62. Duraissamy J, Kaluza KM, Freeman GJ, et al. Dual blockade of PD-1 and CTLA-4 combined with tumor vaccine effectively restores T-cell rejection function in tumors. *Cancer Res* 2013; 73: 3591–3603.

63. Schumacher TN. T-cell-receptor gene therapy. *Nat Rev Immunol* 2002; 2: 512–519.

64. Kochenderfer JN and Rosenberg SA. Treating B-cell cancer with T cells expressing anti-CD19 chimeric antigen receptors. *Nat Rev Clin Oncol* 2013; 10: 267–276.

65. Hassan R, Thomas A, Alewine C, et al. Mesothelin immunotherapy for cancer: ready for prime time? *J Clin Oncol* 2016; 34: 4171–4179.

66. Barrett DM, Liu X, Jiang S, et al. Regimen-specific effects of RNA-modified chimeric antigen receptor T cells in mice with advanced leukemia. *Hum Gene Ther* 2013; 24: 717–727.

67. Di Stasi A, Tey SK, Dotti G, et al. Inducible apoptosis as a safety switch for adoptive cell therapy. *N Engl J Med* 2011; 365: 1673–1683.

68. Beatty GL, Haas AR, Maus MV, et al. Mesothelin-specific chimeric antigen receptor mRNA-engineered T cells induce anti-tumor activity in solid malignancies. *Cancer Immunol Res* 2014; 2: 112–120.

69. Cherkassky L, Morello A, Villena-Vargas J, et al. Human CAR T cells with cell-intrinsic PD-1 checkpoint blockade resist tumor-mediated inhibition. *J Clin Invest* 2016; 126: 3130–3144.

70. Liu X, Ranganathan R, Jiang S, et al. A chimeric switch-receptor targeting PD1 augments the efficacy of second-generation CAR T cells in advanced solid tumors. *Cancer Res* 2016; 76: 1578–1590.

71. Rosenberg SA, Yang JC, Sherry RM, et al. Durable complete responses in heavily pretreated patients with metastatic melanoma using T-cell transfer immunotherapy. *Clin Cancer Res* 2011; 17: 4550–4557.

72. Andersen R, Donia M, Ellebaek E, et al. Long-lasting complete responses in patients with metastatic melanoma after adoptive cell therapy with tumor-infiltrating lymphocytes and an attenuated IL2 regimen. *Clin Cancer Res* 2016; 22: 3734–3745.

73. Ratto GB, Zino P, Mirabelli S, et al. A randomized trial of adoptive immunotherapy with tumor-infiltrating lymphocytes and interleukin-2 versus standard therapy in the postoperative treatment of resected nonsmall cell lung carcinoma. *Cancer* 1996; 78: 244–251.

74. Dudley ME, Wunderlich JR, Shelton TE, et al. Generation of tumor-infiltrating lymphocyte cultures for use in adoptive transfer therapy for melanoma patients. *J Immunother* 2003; 26: 332–342.

75. Ben-Avi R, Farhi R, Ben-Nun A, et al. Establishment of adoptive cell therapy with tumor infiltrating lymphocytes for non-small cell lung cancer patients. *Cancer Immunol Immunother* 2018.
76. Ward-Kavanagh LK, Lin WW, Šedý JR, et al. The TNF receptor superfamily in co-stimulating and co-inhibitory responses. *Immunity* 2016; 44: 1005–1019.

77. Withers DR, Gaspal FM, Bekiaris V, et al.OX40 and CD30 signals in CD4(+) T-cell effector and memory function: a distinct role for lymphoid tissue inducer cells in maintaining CD4(+) T-cell memory but not effector function. *Immunol Rev* 2011; 244: 134–148.

78. Byun M, Ma CS, Akçay A, et al. Inherited human OX40 deficiency underlying classic Kaposi sarcoma of childhood. *J Exp Med* 2013; 210: 1743–1759.

79. Guihot A, Dupin N, Marcelin AG, et al. Low T cell responses to human herpesvirus 8 in patients with AIDS-related and classic Kaposi sarcoma. *J Infect Dis* 2006; 194: 1078–1088.

80. Curti BD, Kovacsovics-Bankowski M, Morris N, et al. OX40 is a potent immune-stimulating target in late-stage cancer patients. *Cancer Res* 2013; 73: 7189–7198.

81. Piconese S, Valzasina B and Colombo MP. OX40 triggering blocks suppression by regulatory T cells and facilitates tumor rejection. *J Exp Med* 2008; 205: 825–839.

82. Bulliard Y, Jolicoeur R, Zhang J, et al. OX40 engagement depletes intratumor Tregs via activating FcγRs, leading to antitumor efficacy. *Immunol Cell Biol* 2014; 92: 475–480.

83. Sadun RE, Hsu WE, Zhang N, et al. Fc-mOX40L fusion protein produces complete remission and enhanced survival in 2 murine tumor models. *J Immunother* 2008; 31: 235–245.

84. Buchan SL, Rogel A and Al-Shamkhani A. The immunobiology of CD27 and OX40 and their potential as targets for cancer immunotherapy. *Blood*. Epub ahead of print 8 November 2017. DOI: 10.1182/blood-2017-07-741025.

85. Agematsu K, Kobata T, Yang FC, et al. CD27/CD70 interaction directly drives B cell IgG and IgM synthesis. *Eur J Immunol* 1995; 25: 2825–2829.

86. Hendriks J, Xiao Y and Borst J. CD27 promotes survival of activated T cells and complements CD28 in generation and establishment of the effector T cell pool. *J Exp Med* 2003; 198: 1369–1380.

87. Yang FC, Agematsu K, Nakazawa T, et al. CD27/CD70 interaction directly induces natural killer cell killing activity. *Immunology* 1996; 88: 289–293.

88. Burris HA, Infante JR, Ansell SM, et al. Safety and activity of varilumab, a novel and first-in-class agonist anti-CD27 antibody, in patients with advanced solid tumors. *J Clin Oncol* 2017; 35: 2028–2036.

89. He LZ, Prostak N, Thomas LJ, et al. Agonist anti-human CD27 monoclonal antibody induces T cell activation and tumor immunity in human CD27-transgenic mice. *J Immunol* 2013; 191: 4174–4183.

90. Wasiuk A, Testa J, Weidlick J, et al. CD27-mediated regulatory T cell depletion and effector T cell co-stimulation both contribute to antitumor efficacy. *J Immunol* 2017; 199: 4110–4123.

91. Kanamaru F, Youngnak P, Hashiguchi M, et al. Costimulation via glucocorticoid-induced TNF receptor in both conventional and CD25+ regulatory CD4+ T cells. *J Immunol* 2004; 172: 7306–7314.

92. Clouthier DL and Watts TH. Cell-specific and context-dependent effects of GITR in cancer, autoimmunity, and infection. *Cytokine Growth Factor Rev* 2014; 25: 91–106.

93. Kim IK, Kim BS, Koh CH, et al. Glucocorticoid-induced tumor necrosis factor receptor-related protein co-stimulation facilitates tumor regression by inducing IL-9-producing helper T cells. *Nat Med* 2015; 21: 1010–1017.

94. Stephens GL, McHugh RS, Whitters MJ, et al. Engagement of glucocorticoid-induced TNFR family-related receptor on effector T cells by its ligand mediates resistance to suppression by CD4+CD25+ T cells. *J Immunol* 2004; 173: 5008–5020.

95. Coe D, Begom S, Addey C, et al. Depletion of regulatory T cells by anti-GITR mAb as a novel mechanism for cancer immunotherapy. *Cancer Immunol Immunothe* 2010; 59: 1367–1377.

96. Lu L, Xu X, Zhang B, et al. Combined PD-1 blockade and GITR triggering induce a potent antitumor immunity in murine cancer models and synergizes with chemotherapeutic drugs. *J Transl Med* 2014; 12: 36.

97. Mitsui J, Nishikawa H, Muraoka D, et al. Two distinct mechanisms of augmented antitumor activity by modulation of immunostimulatory/inhibitory signals. *Clin Cancer Res* 2010; 16: 2781–2791.

98. Vezys V, Penalosa-MacMaster P, Barber DL, et al. 4–1BB signaling synergizes with programmed death ligand 1 blockade to augment CD8 T cell responses during chronic
viral infection. J Immunol 2011; 187: 1634–1642.

99. Willoughby JE, Kerr JP, Rogel A, et al. Differential impact of CD27 and 4–1BB costimulation on effector and memory CD8 T cell generation following peptide immunization. J Immunol 2014; 193: 244–251.

100. Curran MA, Geiger TL, Montalvo W, et al. Systemic 4–1BB activation induces a novel T cell phenotype driven by high expression of Eomesodermin. J Exp Med 2013; 210: 743–755.

101. Chester C, Ambulkar S and Kohrt HE. 4–1BB agonism: adding the accelerator to cancer immunotherapy. Cancer Immunol Immunother 2016; 65: 1243–1248.

102. Kocak E, Lute K, Chang X, et al. Combination therapy with anti-CTL antigen-4 and anti-4–1BB antibodies enhances cancer immunity and reduces autoimmunity. Cancer Res 2006; 66: 7276–7284.

103. Azpilikueta A, Agorreta J, Labiano S, et al. Successful immunotherapy against a transplantable mouse squamous lung carcinoma with anti-PD-1 and anti-CD137 monoclonal antibodies. J Thorac Oncol 2016; 11: 524–536.

104. Weigelin B, Bolaños E, Teijeira A, et al. Focusing and sustaining the antitumor CTL effector killer response by agonist anti-CD137 mAb. Proc Natl Acad Sci U S A 2015; 112: 7551–7556.

105. Beatty GL, Li Y and Long KB. Cancer immunotherapy: activating innate and adaptive immunity through CD40 agonists. Expert Rev Anticancer Ther 2017; 17: 175–186.

106. Bennett SR, Carbone FR, Karamalis F, et al. Help for cytotoxic T-cell responses is mediated by CD40 signalling. Nature 1998; 393: 478–480.

107. Ridge JP, Di Rosa F and Matzinger P. A conditioned dendritic cell can be a temporal bridge between a CD4+ T-helper and a T-killer cell. Nature 1998; 393: 474–478.

108. Schoenberger SP, Toes RE, van der Voort EI, et al. T-cell help for cytotoxic T lymphocytes is mediated by CD40-CD40L interactions. Nature 1998; 393: 480–483.

109. Vonderheide RH, Flaherty KT, Khalil M, et al. Clinical activity and immune modulation in cancer patients treated with CP-870,893, a novel CD40 agonist monoclonal antibody. J Clin Oncol 2007; 25: 876–883.

110. Mangsbo SM, Broos S, Fletcher E, et al. The human agonistic CD40 antibody ADC-1013 eradicates bladder tumors and generates T-cell-dependent tumor immunity. Clin Cancer Res 2015; 21: 1115–1126.

111. Grosso JF, Kelleher CC, Harris TJ, et al. LAG-3 regulates CD8+ T cell accumulation and effector function in murine self- and tumor-tolerance systems. J Clin Invest 2007; 117: 3383–3392.

112. Huang CT, Workman CJ, Flies D, et al. Role of LAG-3 in regulatory T cells. Immunity 2004; 21: 503–513.

113. Huard B, Prigent P, Pagès F, et al. T cell major histocompatibility complex class II molecules down-regulate CD4+ T cell clone responses following LAG-3 binding. Eur J Immunol 1996; 26: 1180–1186.

114. Workman CJ, Rice DS, Dugger KJ, et al. Phenotypic analysis of the murine CD4-related glycoprotein, CD223 (LAG-3). Eur J Immunol 2002; 32: 2255–2263.

115. Workman CJ and Vignali DA. Negative regulation of T cell homeostasis by lymphocyte activation gene-3 (CD223). J Immunol 2005; 174: 688–695.

116. Grosso JF, Goldberg MV, Getnet D, et al. Functionally distinct LAG-3 and PD-1 subsets on activated and chronically stimulated CD8 T cells. J Immunol 2009; 182: 6659–6669.

117. Woos 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: 917–927.

118. Blackburn SD, Shin H, Haining WN, et al. Coregulation of CD8+ T cell exhaustion by multiple inhibitory receptors during chronic viral infection. Nat Immuno 2009; 10: 29–37.

119. Camisaschi C, De Filippo A, Beretta V, et al. Alternative activation of human plasmacytoid DCs in vitro and in melanoma lesions: involvement of LAG-3. J Invest Dermatol 2014; 134: 1893–1902.

120. Li FJ, Zhang Y, Jin GX, et al. Expression of LAG-3 is coincident with the impaired effector function of HBV-specific CD8(+) T cell in HCC patients. Immunol Lett 2013; 150: 116–122.

121. Takaya S, Saito H and Itohuchi M. Upregulation of immune checkpoint molecules, PD-1 and LAG-3, on CD4+ and CD8+ T cells after gastric cancer surgery. Yonago Acta Med 2015; 58: 39–44.
122. He Y, Yu H, Rozeboom L, et al. LAG-3 Protein expression in non-small cell lung cancer and its relationship with PD-1/PD-L1 and tumor-infiltrating lymphocytes. *J Thorac Oncol* 2017; 12: 814–823.

123. He Y, Rivard CJ, Rozeboom L, et al. Lymphocyte-activation gene-3, an important immune checkpoint in cancer. *Cancer Sci* 2016; 107: 1193–1197.

124. Andreae S, Piras F, Burdin N, et al. Maturation and activation of dendritic cells induced by lymphocyte activation gene-3 (CD223). *J Immunol* 2002; 168: 3874–3880.

125. Casati C, Camisaschi C, Rini F, et al. Soluble human LAG-3 molecule amplifies the in vitro generation of type 1 tumor-specific immunity. *Cancer Res* 2006; 66: 4450–4460.

126. Brignone C, Escudier B, Grygar C, et al. A phase I pharmacokinetic and biological correlative study of IMP321, a novel MHC class II agonist, in patients with advanced renal cell carcinoma. *Clin Cancer Res* 2009; 15: 6225–6231.

127. Brignone C, Gutierrez M, Mefti F, et al. First-line chemoinmunotherapy in metastatic breast carcinoma: combination of paclitaxel and IMP321 (LAG-3Ig) enhances immune responses and antitumor activity. *J Transl Med* 2010; 8: 71.

128. Zhu C, Anderson AC, Schubart A, et al. The Tim-3 ligand galectin-9 negatively regulates T helper type 1 immunity. *Nat Immunol* 2005; 6: 1245–1252.

129. Sabatos CA, Chakravarti S, Cha E, et al. Interaction of Tim-3 and Tim-3 ligand regulates T helper type 1 responses and induction of peripheral tolerance. *Nat Immunol* 2003; 4: 1102–1110.

130. Sánchez-Fueyo A, Tian J, Picarella D, et al. Tim-3 inhibits T helper type 1-mediated autoimmune and alloimmune responses and promotes immunological tolerance. *Nat Immunol* 2003; 4: 1093–1101.

131. Anderson AC, Joller N and Kuchroo VK. Lag-3, Tim-3, and TIGIT: co-inhibitory receptors with specialized functions in immune regulation. *Immunity* 2016; 44: 989–1004.

132. Jones RB, Ndhlovu LC, Barbour JD, et al. Tim-3 expression defines a novel population of dysfunctional T cells with highly elevated frequencies in progressive HIV-1 infection. *J Exp Med* 2008; 205: 2763–2779.

133. Sakuishi K, Apetoh L, Sullivan JM, et al. Targeting Tim-3 and PD-1 pathways to reverse T cell exhaustion and restore anti-tumor immunity. *J Exp Med* 2010; 207: 2187–2194.

134. Ngiow SF, von Scheidt B, Akiba H, et al. Anti-TIM3 antibody promotes T cell IFN-γ-mediated antitumor immunity and suppresses established tumors. *Cancer Res* 2011; 71: 3540–3551.

135. Das M, Zhu C and Kuchroo VK. Tim-3 and its role in regulating anti-tumor immunity. *Immunol Rev* 2017; 276: 97–111.

136. Gao X, Zhu Y, Li G, et al. TIM-3 expression characterizes regulatory T cells in tumor tissues and is associated with lung cancer progression. *PLoS One* 2012; 7: e30676.

137. Xu LY, Chen DD, He JY, et al. Tim-3 expression by peripheral natural killer cells and natural killer T cells increases in patients with lung cancer—reduction after surgical resection. *Asian Pac J Cancer Prev* 2014; 15: 9945–9948.

138. Xu L, Huang Y, Tan L, et al. Increased Tim-3 expression in peripheral NK cells predicts a poorer prognosis and Tim-3 blockade improves NK cell-mediated cytotoxicity in human lung adenocarcinoma. *Int Immunopharmacol* 2015; 29: 635–641.

139. Stanietsky N, Simic H, Arapovic J, et al. The interaction of TIGIT with PVR and PVRL2 inhibits human NK cell cytotoxicity. *Proc Natl Acad Sci U S A* 2009; 106: 17858–17863.

140. Yu X, Harden K, Gonzalez LC, et al. The surface protein TIGIT suppresses T cell activation by promoting the generation of mature immunoregulatory dendritic cells. *Nat Immunol* 2009; 10: 48–57.

141. Chan CJ, Andrews DM, McLaughlin NM, et al. DNAM-1/CD155 interactions promote cytokine and NK cell-mediated suppression of poorly immunogenic melanoma metastases. *J Immunol* 2010; 184: 902–911.

142. Chan CJ, Andrews DM and Smyth MJ. Receptors that interact with nectin and nectin-like proteins in the immunosurveillance and immunotherapy of cancer. *Curr Opin Immunol* 2012; 24: 246–251.

143. Chan CJ, Martinet L, Gilfillan S, et al. The receptors CD96 and CD226 oppose each other in the regulation of natural killer cell functions. *Nat Immunol* 2014; 15: 431–438.

144. Kurtulus S, Sakuishi K, Ngiow SF, et al. TIGIT predominantly regulates the immune response via regulatory T cells. *J Clin Invest* 2015; 125: 4053–4062.
145. Johnston RJ, Comps-Agrar L, Hackney J, et al. The immunoreceptor TIGIT regulates antitumor and antiviral CD8(+) T cell effector function. Cancer Cell 2014; 26: 923–937.

146. Chauvin JM, Pagliano O, Fourcade J, et al. TIGIT and PD-1 impair tumor antigen-specific CD8(+) T cells in melanoma patients. J Clin Invest 2015; 125: 2046–2058.

147. Kuśnierczyk P. Killer cell immunoglobulin-like receptor gene associations with autoimmune and allergic diseases, recurrent spontaneous abortion, and neoplasms. Front Immunol 2013; 4: 8.

148. Purdy AK and Campbell KS. Natural killer cells and cancer: regulation by the killer cell Ig-like receptors (KIR). Cancer Biol Ther 2009; 8: 2211–2220.

149. So T, Takenoyama M, Mizukami M, et al. Haplotype loss of HLA class I antigen as an escape mechanism from immune attack in lung cancer. Cancer Res 2005; 65: 5945–5952.

150. Dal Bello MG, Alama A, Coco S, et al. Understanding the checkpoint blockade in lung cancer immunotherapy. Drug Discov Today 2017; 22: 1266–1273.

151. Esendagli G, Bruderek K, Goldmann T, et al. Malignant and non-malignant lung tissue areas are differentially populated by natural killer cells and regulatory T cells in non-small cell lung cancer. Lung Cancer 2008; 59: 32–40.

152. Schneider T, Kimpfler S, Warth A, et al. Foxp3(+) regulatory T cells and natural killer cells distinctly infiltrate primary tumors and draining lymph nodes in pulmonary adenocarcinoma. J Thorac Oncol 2011; 6: 432–438.

153. Carrega P, Morandi B, Costa R, et al. Natural killer cells infiltrating human non-small cell lung cancer are enriched in CD56 bright CD16(-) cells and display an impaired capability to kill tumor cells. Cancer 2008; 112: 863–875.

154. He Y, Bunn PA, Zhou C, et al. KIR 2D (L1, L3, L4, S4) and KIR 3DL1 protein expression in non-small cell lung cancer. Oncotarget 2016; 7: 82104–82111.

155. Romagné F, André P, Spee P, et al. Preclinical characterization of 1–7F9, a novel human anti-KIR receptor therapeutic antibody that augments natural killer-mediated killing of tumor cells. Blood 2009; 114: 2667–2677.

156. Kohrt HE, Thielen A, Marabelle A, et al. Anti-KIR antibody enhancement of anti-lymphoma activity of natural killer cells as monotherapy and in combination with anti-CD20 antibodies. Blood 2014; 123: 678–686.

157. Guillerey C, Huntington ND and Smyth MJ. Targeting natural killer cells in cancer immunotherapy. Nat Immunol 2016; 17: 1025–1036.

158. Wieten L, Mahaweni NM, Voorter CE, et al. Clinical and immunological significance of HLA-E in stem cell transplantation and cancer. Tissue Antigens 2014; 84: 523–535.

159. Talebian Yazdi M, van Riet S, van Schadewijk A, et al. The positive prognostic effect of stromal CD8(+) tumor-infiltrating T cells is restrained by the expression of HLA-E in non-small cell lung carcinoma. Oncotarget 2016; 7: 3477–3488.

160. Nowak EC, Lines JL, Varn FS, et al. Immunoregulatory functions of VISTA. Immunol Rev 2017; 276: 66–79.

161. Lines JL, Pantazi E, Mak J, et al. VISTA is an immune checkpoint molecule for human T cells. Cancer Res 2014; 74: 1924–1932.

162. Le Mercier I, Chen W, Lines JL, et al. VISTA regulates the development of protective antitumor immunity. Cancer Res 2014; 74: 1933–1944.

163. Wang L, Rubinstein R, Lines JL, et al. VISTA, a novel mouse Ig superfamily ligand that negatively regulates T cell responses. J Exp Med 2011; 208: 577–592.

164. Liu J, Yuan Y, Chen W, et al. Immune-checkpoint proteins VISTA and PD-1 nonredundantly regulate murine T-cell responses. Proc Natl Acad Sci U S A 2015; 112: 6682–6687.

165. Powderly J, Patel MR, Lee JJ, et al. Abstract 1141PD: CA-170, a first in class oral small molecule dual inhibitor of immune checkpoints PD-L1 and VISTA, demonstrates tumor growth inhibition in pre-clinical models and promotes T cell activation in Phase 1 study. Annals of Oncology 2017; 28: v403–v427.

166. Théate I, van Baren N, Pilotte L, et al. Extensive profiling of the expression of the indoleamine 2,3-dioxygenase 1 protein in normal and tumoral human tissues. Cancer Immunol Res 2015; 3: 161–172.

167. Brochez L, Chevolet I and Kruse V. The rationale of indoleamine 2,3-dioxygenase inhibition for cancer therapy. Eur J Cancer 2017; 76: 167–182.

168. Munn DH, Sharma MD, Baban B, et al. GCN2 kinase in T cells mediates proliferative...
arrest and anergy induction in response to indoleamine 2,3-dioxygenase. *Immunity* 2005; 22: 633–642.

169. Nguyen NT, Kimura A, Nakahama T, et al. Aryl hydrocarbon receptor negatively regulates dendritic cell immunogenicity via a kynurenine-dependent mechanism. *Proc Natl Acad Sci U S A* 2010; 107: 19961–19966.

170. Taylor MW and Feng GS. Relationship between interferon-gamma, indoleamine 2,3-dioxygenase, and tryptophan catabolism. *FASEB J* 1991; 5: 2516–2522.

171. Zhai L, Spranger S, Binder DC, et al. Molecular pathways: targeting IDO1 and other tryptophan dioxygenases for cancer immunotherapy. *Clin Cancer Res* 2015; 21: 5427–5433.

172. Suzuki Y, Suda T, Furuhashi K, et al. Increased serum kynurenine/tryptophan ratio correlates with disease progression in lung cancer. *Lung Cancer* 2010; 67: 361–365.

173. Creelan BC, Antonia S, Bepler G, et al. Indoleamine 2,3-dioxygenase activity and clinical outcome following induction chemotherapy and concurrent chemoradiation in Stage III non-small cell lung cancer. *Oncoimmunology* 2013; 2: e23428.

174. Liu X, Shin N, Koblish HK, et al. Selective inhibition of IDO1 effectively regulates mediators of antitumor immunity. *Blood* 2010; 115: 3520–3530.

175. Beatty GL, O'Dwyer PJ, Clark J, et al. First-in-human phase I study of the oral inhibitor of indoleamine 2,3-dioxygenase-1 epacadostat (INCB024360) in patients with advanced solid malignancies. *Clin Cancer Res* 2017; 23: 3269–3276.

176. Chevrotet I, Speeckaert R, Schreuer M, et al. Characterization of the in vivo immune network of IDO, tryptophan metabolism, PD-L1, and CTLA-4 in circulating immune cells in melanoma. *Oncoimmunology* 2015; 4: e982382.

177. Gangadhar T, Schneider B, Bauer T, et al. Efficacy and safety of epacadostat plus pembrolizumab treatment of NSCLC: preliminary phase I/II results of ECHO-202/KEYNOTE-037. *J Clin Oncol* 2017; 35(Suppl.): abstract 9014.

178. Long GV, Dummer R, Hamid, et al. Epacadostat (E) plus pembrolizumab (P) versus pembrolizumab alone in patients (pts) with unresectable or metastatic melanoma: results of the phase 3 ECHO-301/KEYNOTE-252 study. *J Clin Oncol* 2018; 36(Suppl.): abstract 108.

179. Soliman H, Anotonia S, Sullivan D, et al. Overcoming tumor antigen anergy in human malignancies using the novel indoleamine 2,3-dioxygenase (IDO) enzyme inhibitor, 1-methyl-D-tryptophan (IMT). *J Clin Oncol* 2009; 27(15 Suppl.): abstract 3004.

180. Muller AJ, DuHaddaway JB, Donover PS, et al. Inhibition of indoleamine 2,3-dioxygenase, an immunoregulatory target of the cancer suppression gene Bin1, potentiates cancer chemotherapy. *Nat Med* 2005; 11: 312–319.

181. Soliman HH, Jackson E, Neuger T, et al. A first in man phase I trial of the oral immunomodulator, indoximod, combined with docetaxel in patients with metastatic solid tumors. *Oncotarget* 2014; 5: 8136–8146.

182. Malhotra J, Jabbour SK and Aisner J. Current state of immunotherapy for non-small cell lung cancer. *Transl Lung Cancer Res* 2017; 6: 196–211.

183. Morris J, Rossi G, Harrold N, et al. Potential chemo-sensitization effect of tergenpumatucel-L immunotherapy in treated patients with advanced non-small cell lung cancer (NSCLC). *J Clin Oncol* 2013; 31(15 Suppl.): abstract 8094.

184. Iversen TZ, Engell-Noerrengaard L, Ellebaek E, et al. Long-lasting disease stabilization in the absence of toxicity in metastatic lung cancer patients vaccinated with an epitope derived from indoleamine 2,3 dioxygenase. *Clin Cancer Res* 2014; 20: 221–232.

185. Stagg J and Smyth MJ. Extracellular adenosine triphosphate and adenosine in cancer. *Oncogene* 2010; 29: 5346–5358.

186. Ohta A, Gorelik E, Prasad SJ, et al. A2A adenosine receptor protects tumors from antitumor T cells. *Proc Natl Acad Sci U S A* 2006; 103: 13132–13137.

187. Kong T, Westerman KA, Faigle M, et al. HIF-dependent induction of adenosine A2B receptor in hypoxia. *FASEB J* 2006; 20: 2242–2250.

188. Deaglio S, Dwyer KM, Gao W, et al. Adenosine generation catalyzed by CD39 and CD73 expressed on regulatory T cells mediates immune suppression. *J Exp Med* 2007; 204: 1257–1265.

189. Inoue Y, Yoshimura K, Kurabe N, et al. Prognostic impact of CD73 and A2A adenosine receptor expression in non-small-cell lung cancer. *Oncotarget* 2017; 8: 8738–8751.
190. Blay J, White TD and Hoskin DW. The extracellular fluid of solid carcinomas contains immunosuppressive concentrations of adenosine. *Cancer Res* 1997; 57: 2602–2605.

191. Mediavilla-Varela M, Luddy K, Noyes D, *et al*. Antagonism of adenosine A2A receptor expressed by lung adenocarcinoma tumor cells and cancer associated fibroblasts inhibits their growth. *Cancer Biol Ther* 2013; 14: 860–868.

192. Young A, Mittal D, Stagg J, *et al*. Targeting cancer-derived adenosine: new therapeutic approaches. *Cancer Discov* 2014; 4: 879–888.

193. Hay CM, Sult E, Huang Q, *et al*. Targeting CD73 in the tumor microenvironment with MEDI9447. *Oncoimmunology* 2016; 5: e1208875.

194. Mediavilla-Varela M, Castro J, Chiappori A, *et al*. A novel antagonist of the immune checkpoint protein adenosine A2a receptor restores tumor-infiltrating lymphocyte activity in the context of the tumor microenvironment. *Neoplasia* 2017; 19: 530–536.

195. Emens L, Powderly J, Fong L, *et al*. Abstract CT119: CPI-444, an oral adenosine A2a receptor (A2aR) antagonist, demonstrates clinical activity in patients with advanced solid tumors. *Cancer Res* 2017; 77: CT119.

196. Bronte V and Zanovello P. Regulation of immune responses by L-arginine metabolism. *Nat Rev Immunol* 2005; 5: 641–654.

197. Rodriguez PC, Zea AH, DeSalvo J, *et al*. L-arginine consumption by macrophages modulates the expression of CD3 zeta chain in T lymphocytes. *J Immunol* 2003; 171: 1232–1239.

198. Rodriguez PC, Quiceno DG, Zabaleta J, *et al*. Arginase I production in the tumor microenvironment by mature myeloid cells inhibits T-cell receptor expression and antigen-specific T-cell responses. *Cancer Res* 2004; 64: 5839–5849.

199. Ostuni R, Kratochvill F, Murray PJ, *et al*. Macrophages and cancer: from mechanisms to therapeutic implications. *Trends Immunol* 2015; 36: 229–239.

200. Kuang DM, Zhao Q, Peng C, *et al*. Activated monocytes in peritumoral stroma of hepatocellular carcinoma foster immune privilege and disease progression through PD-L1. *J Exp Med* 2009; 206: 1327–1337.

201. Ruffell B, Chang-Strachan D, Chan V, *et al*. Macrophage IL-10 blocks CD8+ T cell-dependent responses to chemotherapy by suppressing IL-12 expression in intratumoral dendritic cells. *Cancer Cell* 2014; 26: 623–637.

202. Emmerich J, Mumm JB, Chan IH, *et al*. IL-10 directly activates and expands tumor-resident CD8(+) T cells without de novo infiltration from secondary lymphoid organs. *Cancer Res* 2012; 72: 3570–3581.

203. Naing A, Papadopoulos KP, Autio KA, *et al*. Safety, antitumor activity, and immune activation of pegylated recombinant human interleukin-10 (AM0010) in patients with advanced solid tumors. *J Clin Oncol* 2016; 34: 3562–3569.

204. Cannarile MA, Weisser M, Jacob W, *et al*. Colony-stimulating factor 1 receptor (CSF1R) inhibitors in cancer therapy. *J Immunother Cancer* 2017; 5: 53.

205. Ries CH, Cannarile MA, Hoves S, *et al*. Targeting tumor-associated macrophages with anti-CSF-1R antibody reveals a strategy for cancer therapy. *Cancer Cell* 2014; 25: 846–859.

206. Almatroodi SA, McDonald CF, Darby IA, *et al*. Characterization of M1/M2 tumour-associated macrophages (TAMs) and Th1/Th2 cytokine profiles in patients with NSCLC. *Cancer Microenviron* 2016; 9: 1–11.

207. Wang R, Lu M, Zhang J, *et al*. Increased IL-10 mRNA expression in tumor-associated macrophage correlated with late stage of lung cancer. *J Exp Clin Cancer Res* 2011; 30: 62.

208. Büttner R, Gosney JR, Skov BG, *et al*. Programmed death-ligand 1 immunohistochemistry testing: a review of analytical assays and clinical implementation in non-small cell lung cancer. *JAMA Oncol* 2017; 35: 3867–3876.

209. McLaughlin J, Han G, Schalper KA, *et al*. Quantitative assessment of the heterogeneity of PD-L1 expression in non-small cell lung cancer. *JAMA Oncol* 2016; 2: 46–54.

210. Lawrence MS, Stojanov P, Polak P, *et al*. Mutational heterogeneity in cancer and the search for new cancer-associated genes. *Nature* 2013; 499: 214–218.

211. Kowantetz M, Zou W, McCleland M, *et al*. MA 05.09 Pre-existing immunity measured by teff gene expression in tumor tissue is associated with atezolizumab efficacy in NSCLC. *J Thorac Oncol* 2017; 12: S1817–S1818.