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The role of immunotherapy in treatment of non-small-cell lung cancer (NSCLC) has been gaining interest over the past few years. This has been driven primarily by promising results from trials evaluating antagonist antibodies that target co-inhibitory immune checkpoints expressed on tumor cells and immune cells within the tumor microenvironment. Immune checkpoints exist to dampen or terminate immune activity to guard against autoimmunity and allow for self-tolerance. However, tumors can take advantage of these immune checkpoint pathways to evade destruction. Antibodies that block inhibitory checkpoints, such as anti-CTLA-4, anti-PD1 and anti-PD-L1 antibodies have demonstrated delayed tumor growth and increased survival. Novel therapies are now investigating combining checkpoint inhibitors with chemotherapy, targeted therapy, radiation and vaccines to produce synergistic antitumor activity.

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Lung cancer is the leading cause of cancer-related deaths in the United States and worldwide [1]. Non-small-cell lung cancer (NSCLC) accounts for about 85% of all lung cancers and is often diagnosed at an advanced stage with poor overall prognosis [2]. Mainstay treatment for metastatic NSCLC is platinum-based doublet chemotherapy with a median survival of about 10 months [3]. With the addition of bevacizumab, an anti-angiogenesis agent, to chemotherapy, median survival has improved to 1 year [3,4].

A subgroup of patients with NSCLC have specific mutations in the epidermal growth factor receptor (EGFR) or anaplastic lymphoma kinase (ALK) gene that can serve as potential targets for therapy. Somatic activating mutations in the kinase domain of EGFR have been identified in 10–15% of patients of Caucasian ethnicity and 30–50% of patients in Asia [2,5,6]. The ALK gene rearrangement is found in approximately 4–5% of all NSCLC, including both Caucasian and Asian populations [7–10]. Tyrosine kinase inhibitors (TKIs), such as gefitinib and erlotinib, and small molecule ALK inhibitors such as crizotinib, have resulted in higher response rates and prolonged survival. However, the vast majority of patients will eventually relapse within 1 year, illustrating the need for additional, more effective therapies [2,7].

Immune checkpoint overview
Recently, there has been a renewed interest in immunotherapies for the treatment of NSCLC, especially with promising results in patients with advanced disease who have progressed on multiple lines of treatment.

T-cell immunity is mediated by interactions between antigen-presenting cells
(APCs) and T cells, regulated by co-stimulatory and inhibitory signals. For T-cell response to occur, co-stimulatory signals must be engaged by their cognate ligands as illustrated in Figure 1. Co-stimulatory and inhibitory receptors often bind to the same ligand with varying kinetics [11]. Co-stimulatory receptors are expressed on naive and resting T cells while inhibitory receptors are upregulated after T-cell activation to maintain self-tolerance and limit tissue damage during immune responses [11,12].

Immune checkpoints are crucial for downregulation of the immune system to prevent autoimmunity [13,14]. However, the tumor microenvironment has been shown to be composed of dysfunctional immune cells and stromal host cells that exploit immunoregulatory pathways to evade immunity [15]. Inhibitory ligands and receptors are commonly overexpressed on tumor or in the tumor microenvironment and are targetable with antagonist antibodies [11]. Major mechanisms of immune evasion by tumors that can be clinically targeted include the CTLA-4 and PD-1 pathways [15]. Properties of the most common anti-CTLA-4, PD-1, and PD-L1 antibodies are shown in Table 1, including type of antibody and binding affinity. Inhibition of these pathways has been shown in preclinical models to improve intratumoral immune responses [13,16,17]. Better understanding of these pathways and immune invasion is essential for development of effective treatments.

**Anti-CTLA-4 pathway**

CTLA-4 and CD28 are immunoglobulin (Ig) co-receptors that bind to CD80 (B7–1) and CD86 (B7–2) [18–20]. CD80 binds CTLA-4 with greater affinity than CD28, Kd values of approximately 12 and 200 nM, respectively [21]. CD28 co-stimulation is required for T cell activation to most peptide antigens and results in activation of NFAT and NF-κB [18,22–27].

In contrast, CTLA-4 opposes the actions of CD28-mediated co-stimulation and functions as an inhibitor of T-cell response to regulate T cell proliferation, allowing for self-tolerance. [28,29] CTLA-4 has been shown to downregulate AKT phosphorylation through recruitment of two phosphatases, SHP2 and PP2A, to dampen T cell activation and decrease production of NF-κB, IL-2 and cytokines as shown in Figure 2 [30–33]. CTLA-4 has also been shown to regulate tumor immunity through regulatory T cells (Tregs), which express high levels of surface CTLA-4, and therefore suppressing activation and expansion of effector cells [34]. Blocking CTLA-4 or depletion of Tregs have been found to increase immune response, with increase in antigen-specific T follicular helper (Tfh) cells, plasma and memory B cells after vaccination and

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**Figure 1.** Immune priming occurs in the lymph node and requires a secondary co-stimulatory signal to activate T-cell response, resulting in production of granzymes, perforin, IFN-γ and activation of the PI3 kinase pathway, to allow for T-cell proliferation and destruction of pathogens [101].
enhance secondary immune responses \[^{[35,36]}\]. Tregs have been described to be on tumor cells, and blocking Treg function through anti-CTLA-4 antibodies may be an effective mechanism to enhance antitumor activity \[^{[34]}\].

**Ipilimumab**

Ipilimumab is a fully human immunoglobulin G1 anti-CTLA-4 monoclonal antibody \[^{[34]}\]. Activity of ipilimumab was described in melanoma in a Phase III trial evaluating ipilimumab plus glycoprotein 100 (gp100) peptide vaccine, ipilimumab alone, or gp100 alone in patients with stage III or IV melanoma. They described improved overall survival (OS) with ipilimumab alone compared with gp100 alone (HR for survival = 0.66, \(p = 0.003\)) and also an improved OS in ipilimumab plus gp100 compared with gp100 alone (HR = 0.68, \(p < 0.001\)) \[^{[34,37]}\]. Ipilimumab was US FDA approved for treatment of late stage melanoma in 2011.

Ipilimumab has also been shown to be effective in NSCLC. A Phase II study evaluated chemotherapy-naive patients with NSCLC \((n = 204)\) who were randomly assigned to 1:1:1 to receive paclitaxel (175 mg/m\(^2\)) and carboplatin (area under the curve, 6) with concurrent ipilimumab (four doses of ipilimumab plus paclitaxel and carboplatin), phased ipilimumab (two doses of placebo plus paclitaxel and carboplatin), phased ipilimumab (two doses of placebo plus paclitaxel and carboplatin followed by four doses of ipilimumab plus paclitaxel and carboplatin), or placebo control (up to 6 doses of placebo plus paclitaxel and carboplatin). The study showed improved immune-related progression free survival (irPFS) for phased ipilimumab versus the control (hazard ratio [HR]: 0.72; \(p = 0.05\)) but not for concurrent ipilimumab (HR: 0.81; \(p = 0.13\)). Median irPFS for phased ipilimumab, concurrent ipilimumab and control groups were 5.7, 5.5 and 4.6 months, respectively; median progression free survival (PFS) were 5.1, 4.1 and 4.2 months, respectively; immune-related best overall response rate were 32, 21 and 18%, respectively; best overall response rate were 32, 21 and 14%, respectively, and a median OS of 12.2, 9.7 and 8.3 months. Overall rates of grade 3 and 4 immune related adverse events were 15% for phased ipilimumab, 20% for concurrent ipilimumab and 6% for the control group. The most common adverse events were liver function enzyme abnormalities, hematologic abnormalities (thrombocytopenia, anemia and neutropenia), fatigue, alopecia, nausea, peripheral neuropathy, rash, pruritus and diarrhea. This trial showed that combination of phased ipilimumab with paclitaxel and carboplatin, but not concurrent, improved irPFS. Interestingly, subset analysis appeared to show improved efficiency with phased ipilimumab for squamous histology, without apparent benefit for nonsquamous histology, indicating the need for additional investigation in a larger clinical trial \[^{[38]}\].

**Tremelimumab**

Tremelimumab is a fully human IgG2 anti-CTLA4 antibody, and has been most well studied in melanoma. A Phase III study examined tremelimumab at 15 mg/kg every 3 months compared with standard chemotherapy in patients with metastatic melanoma without brain metastases. At interim analysis, there was a nonstatistically significant improvement in survival favoring tremelimumab \((p = 0.14)\) \[^{[39,40]}\].

Tremelimumab in NSCLC was studied in a randomized Phase II clinical trial comparing tremelimumab as maintenance therapy with best supportive care in patients with good performance status who previously received platinum-based chemotherapy. Patients assigned to the treatment arm received tremelimumab 15 mg/kg IV every 90 days until disease progression. Results showed that treatment with tremelimumab did not result in a superior PFS. However, there were 2 (4.8%) partial responses seen only in the investigational arm which may support additional studies \[^{[41]}\].

**PD-1/PDL-1 pathway**

A major mechanism of immune escape has been described by the B7-H1/PD-1 pathway \[^{[15]}\]. PD-1 is
expressed by activated T cells and is a key immune checkpoint receptor that mediates immunosuppression. It attenuates T cell receptor signaling by binding to its ligands PD-L1 (B7-H1) and PD-L2 (B7-DC) [42].

PDL-1 expression on tumor and tumor infiltrating immune cells is thought to be primarily induced by Th1 cytokines (in particular, interferon-γ) released by infiltrating tumor immune cells [43, 44]. PD-1 signaling has been found to recruit SHP-2, which mediates decreased T-cell receptor signaling possibly through inhibition of the AKT pathway [30, 45–48].

Clinical trials have produced promising results evaluating antagonistic antibodies that target the co-inhibitory immune checkpoint, PD-1 expressed on activated antitumor T cells and its primary ligand, PD-L1 expressed on tumor cells and immune cells within the tumor microenvironment. Targeting this pathway can be categorized as directed against PD-1 such as nivolumab and pembrolizumab, or targeted against PD-L1, such as atezolizumab (MPDL3280A) and durvalumab (MEDI4736). Response rates for these compounds have been consistently over 20% across all trials, which has led to large ongoing Phase III trials, and recent FDA approval of nivolumab for metastatic squamous cell lung cancer [49].

**Anti-PD1**

**Nivolumab**

Nivolumab is a fully human IgG4 monoclonal antibody against PD-1 [50]. Nivolumab was investigated with the CheckMate -017 trial, which was a Phase III, open-label, randomized, multicenter study of nivolumab compared with docetaxel as second-line in patients with squamous cell lung cancer (NCT01642004). This trial was stopped early after interim analysis showed superior overall survival of patients in the nivolumab arm compared with control. In this study, patients with metastatic squamous cell lung cancer, who progressed on platinum doublet-based chemotherapy, were randomized to receive nivolumab (3 mg/kg every 2 weeks) (n = 135) or standard of care, docetaxel (75 mg/m² IV every 3 weeks; n = 137). During interim analysis in January 2015, the median OS was 9.2 months in the nivolumab arm (95% CI: 7.3–13.3) and 6 months in the docetaxel arm (95% CI: 5.1–7.3). The hazard ratio was 0.59 (95% CI: 0.44–0.79; p = 0.00025), translating to a 41% lower risk of death with nivolumab compared with docetaxel. One year overall survival rate was 42% (95% CI: 34–50) with nivolumab, compared with 24% (95% CI: 17–31) with docetaxel. Response rate in the nivolumab arm was 20% compared with 9% in the docetaxel arm (p = 0.008). Median progression free survival was 3.5 months with nivolumab.
and 2.8 months with docetaxel, resulting in a hazard ratio for death or disease progression of 0.62 (95% CI: 0.47–0.81; p < 0.001). The improved overall survival led to early termination of the trial and FDA approval of nivolumab as second line therapy for metastatic squamous lung cancer. This trial also evaluated tumor PD-L1 expression and quantified levels as ≥1, <1, ≥5, <5 and ≥10% and found that tumor PD-L1 status was not prognostic or predictive of efficacy [51,52].

A Phase III, open-label, randomized study (CheckMate-057) examined 582 patients with nonsquamous NSCLC, who had disease recurrence or progression during/after one prior line of platinum based doublet chemotherapy (NCT01673867). Patients were randomized to receive nivolumab 3 mg/kg IV every 2 weeks (n = 292) or docetaxel 75 mg/m² IV every three weeks (n = 290). The primary endpoint was overall survival with secondary endpoints being objective response rate (ORR) and PFS. The study was ended early in April 2015 due to survival advantage observed in the nivolumab arm. Nivolumab showed better OS and response rate. Median OS in the nivolumab group was 12.2 months (95% CI: 9.7–15.0) compared with 9.4 months (95% CI: 8.1–10.7) in the docetaxel group (HR = 0.73; 96% CI: 0.59–0.89; p = 0.002), and response rate was 19.2% with nivolumab versus 12.4% with docetaxel (p = 0.02335). One year overall survival was 51% (95% CI: 45–56) with nivolumab compared with 39% (95% CI: 33–45) with docetaxel. At 18 months, the overall survival was 39% (95% CI: 34–45) with nivolumab, compared with 23% (95% CI: 19–28) with docetaxel. PD-L1 expression was quantified as ≥1, <1, ≥5, <5 and ≥10%, but in contrast to the CheckMate-017 study in squamous NSCLC, this study showed that PD-L1 expression was associated with improved efficacy across all predefined cutpoints of 1, 5 and 10%. Grade 3–5 drug-related adverse events were less common in the nivolumab arm, 10.5% with nivolumab versus 17% of patients experienced peripheral IFN-γ stimulated cytokines, CXCL9 and CXCL10 were observed. 17% of patients experienced grade 3–4 treatment related adverse events, including fatigue (4%), diarrhea (3%) and pneumonitis (3%). This study demonstrates that nivolumab is clinically efficacious with acceptable safety profile [56].

Nivolumab as monotherapy in treatment-naive patients with NSCLC is also being evaluated in an ongoing Phase I study (NCT01454102). As of interim analysis, 52 patients with squamous or nonsquamous advanced NSCLC received nivolumab 3 mg/kg IV every 2 weeks until progression of disease or unacceptable toxicity. ORR was 21% with 3 confirmed complete responses. Response was also evaluated by tumor PD-L1 expression (PD-L1+ was defined as ≥5% tumor cells expressing PD-L1). Responses were observed in patients who were both PD-L1+ and PD-L1−, with ORR higher in patients with PD-L1+ tumors, 31% compared with 10% [57].

**Pembrolizumab**

Pembrolizumab (MK-3475) is a humanized monoclonal IgG4 antibody that is potent and highly selective against PD-1. It was approved by the FDA in 2014 for treatment of metastatic melanoma and was granted accelerated approval on 2 October 2015 as second line treatment for PD-L1 positive metastatic NSCLC. The FDA recently approved a PD-L1 immunohistochemistry 22C3 pharmDx test developed by Dako to aid in identifying patients for treatment with pembrolizumab [58].

The safety and tolerability of pembrolizumab in NSCLC was evaluated in a Phase I study (KEYNOTE-001) that enrolled 495 patients, including 394 patients who had progressed on prior lines of treatment and 101 patients who have never received treatment. Patients were treated with pembrolizumab at dose of either 2 mg or 10 mg per kilogram of body weight every 3 weeks or 10 mg per kilogram every 2 weeks (NCT01295827). The overall response rate was 19.4% (95% CI: 16.0–23.2). Of the previously treated patients, response rate was 18.0% (95% CI: 14.4–22.2). Response rate was 24.8% (95% CI: 16.70–34.3) in patients who had never been treated. Response rates were similar regardless of dose, schedule and histologic analysis. Median duration of response was 12.5 months (range: 1.0–23.3) in all patients with median duration of progression-free survival of 3.7 months (95% CI: 2.9–4.1). Median progression-free survival for all patients was 3.7 months (95% CI: 2.9–4.1), with 3.0 months (95% CI: 2.2–4.0) for previously treated patients, and 6 months (95% CI: 4.1–8.6) for previously untreated patients. The most common drug related adverse events were fatigue (19.4%), pruritus (10.7%), decreased appetite (10.5%), rash (9.7%), arthralgia (9.1%), diarrhea (8.1%), nausea (7.5%) and hypothyroidism (6.9%) [59–61].

This study further examined PD-L1 as a potential biomarker of response and showed that tumor cells with at least 50% PD-L1 expression had improved
<table>
<thead>
<tr>
<th>Drug</th>
<th>NCT</th>
<th>Study</th>
<th>Population</th>
<th>PD-L1</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nivolumab</td>
<td>NCT01642004</td>
<td>CheckMate-017</td>
<td>Squamous cell lung cancer</td>
<td>Quantified levels as ≥1%, &lt;1%, ≥5%, &lt;5% and ≥10% (tumor cell)</td>
<td>PD-L1 not prognostic or predictive of efficacy</td>
</tr>
<tr>
<td></td>
<td>NCT01673867</td>
<td>CheckMate-057</td>
<td>Nonsquamous cell lung cancer</td>
<td>Quantified levels as ≥1%, &lt;1%, ≥5%, &lt;5% and ≥10% (tumor cell)</td>
<td>PD-L1 expression associated with improved efficacy</td>
</tr>
<tr>
<td>Pembrolizumab</td>
<td>NCT01295827</td>
<td>KeyNote-001</td>
<td>Both squamous and nonsquamous lung cancer</td>
<td>Proportion score of &lt;1%, 1–49% and &gt;50% (tumor cell)</td>
<td>PD-L1 expression of at least 50% associated with increased response rate.</td>
</tr>
<tr>
<td></td>
<td>NCT01905657</td>
<td>KeyNote-010</td>
<td>Both squamous and nonsquamous lung cancer</td>
<td>Proportion score &gt;1% considered PD-L1+. &gt;50% strongly PD-L1+ (tumor cell)</td>
<td>Treatment with pembrolizumab associated with longer OS compared with docetaxel for PD-L1 &gt;1% and &gt;50%</td>
</tr>
<tr>
<td>Atezolizumab</td>
<td>NCT01375842</td>
<td></td>
<td>Incurable NSCLC, melanoma, renal, colorectal, gastric, head and neck</td>
<td>IHC of 0, 1, 2, or 3 if &lt;1%, 1–5%, 5–10%, or ≥10% of cells per area were PD-L1 positive (tumor and immune cell)</td>
<td>Tumor infiltrating immune cell PD-L1 associated with response in NSCLC. Tumor cell PD-L1 was not associated with statistically significant response across all tumor types</td>
</tr>
<tr>
<td></td>
<td>NCT01846416</td>
<td>FIR</td>
<td>Both squamous and nonsquamous lung cancer</td>
<td>IHC of 0, 1, 2, or 3 if &lt;1%, 1–5%, 5–10%, or ≥10% of cells per area were PD-L1 positive (tumor and immune cell)</td>
<td>Highest ORR observed in patients with PD-L1 TC3 or IC3</td>
</tr>
<tr>
<td></td>
<td>NCT01903993</td>
<td>POPLAR</td>
<td>Both squamous and nonsquamous lung cancer</td>
<td>IHC of 0, 1, 2, or 3 if &lt;1%, 1–5%, 5–10%, or ≥10% of cells per area were PD-L1 positive (tumor and immune cell)</td>
<td>Increasing TC or IC PD-L1 expression associated with improved OS, PFS and ORR compared with docetaxel</td>
</tr>
</tbody>
</table>

PD-L1 expression is quantified differently in studies, highlighting the need for standardized assays and methods of quantifying PD-L1. IC: Immune cell; TC: Tumor cell. [51,53,54,62,64].
response to pembrolizumab. Membranous PD-L1 expression in at least 50% of tumor cells (proportion score ≥50%) was selected as the cut-off. The response rate was 45.2% (95% CI: 33.5–57.3) in the 73 patients with proportion score of at least 50%, which included 50.0% (95% CI: 24.7–75.3) of patients who had not received prior treatment and 43.9% of patients who had progressed on prior lines of treatment. The response rate of patients with proportion score of at least 50% was statistically significantly better than the group with proportion score 1–49% and <1% in both patients who had previous treatment (p < 0.001) and for patients who never received prior lines of treatment (p = 0.01). The prevalence of PD-L1 expression was examined in 1143 patients who were screened, 824 samples were available for analysis. 23.2% of patients had a proportion score of at least 50%, 37.6% of patients had a score of 1–49% and 39.2% of patients had a score <1% [59].

Another pivotal study of pembrolizumab was Keynote-010, which examined pembrolizumab at the 2 mg/kg FDA-approved dose and higher 10 mg/kg investigational dose, each given every 3 weeks, compared with docetaxel, in PD-L1 positive patients who have progressed on prior lines of therapy (NCT01905657). PD-L1 positivity was defined as tumor proportion score (TPS) of 1% or greater, and strong PD-L1 positivity was classified as TPS of 50% or greater. Pembrolizumab at both doses was associated with longer OS and superior PFS compared with docetaxel in TPS score >50% and greater than 1% [62].

The role of pembrolizumab in treatment of CNS metastasis was evaluated in an ongoing Phase II trial with two separate arms, one for advanced NSCLC and one for metastatic melanoma (NCT02085070). Patients with ≥1 untreated or progressing brain metastases with pretumor biopsy showing PD-L1 expression were enrolled to receive pembrolizumab 10 mg/kg IV every 2 weeks. As of December 2014, ten patients with NSCLC were enrolled, with nine patients evaluable for response. Brain metastasis response rate was 44% (4/9 partial responses). 34% (3/9) patients had progression of disease. Systemic response rate was 34% (3/9). One patient responded systemically but had progression of disease in the brain. This study showed that pembrolizumab has promising clinical activity in CNS disease [63].

**Anti-PDL-1**

**Atezolizumab (MPDL 3280A)**

MPDL3280A, most recently named atezolizumab, is a high-affinity specific human IgG1 antibody against PD-L1 and therefore prevents its binding to ligands PD-1 and B7–1 [64]. However, unlike PD-1 inhibitors, PD-L1 inhibition will allow PD-1 to bind to its alternative ligand PD-L2, which has been described to maintain tolerance in the lung, and may decrease risk for pneumonitis [61,64–66].

In a single arm Phase I study, a total of 277 patients with advanced incurable cancer (NSCLC, melanoma, renal cell carcinoma, colorectal cancer, gastric cancer and head and neck squamous cell carcinoma) were enrolled to receive atezolizumab every 3 weeks intravenously (NCT01375842). One hundred and seventy-five patients were evaluable. Confirmed responses (complete and partial responses) was 18% (32 of 175 patients) across all tumor types. Response rate of 21% (11 of 53 patients) was observed for patients with NSCLC. Patients with melanoma had the highest response rate of 26% (11 of 43 patients). Median PFS of all patients in the study was 18 weeks. Exploratory analysis revealed a potential trend of better response to atezolizumab in former/current smokers versus never smokers (46% [11 of 26] versus 10% [1 of 10] respectively; p = 0.422). Most drug-related adverse events were grade 1–2 and did not require treatment. Thirty-five patients (13%) experienced treatment related grade 3–4 toxicities, and 3 patients (1%) experienced immune-related grade 3–4 adverse events. There were no cases of grade 3–5 pneumonitis. The most common treatment related adverse events were fatigue (24%), decreased appetite (12%), nausea (12%), pyrexia (11%), diarrhea (11%) and rash (11%) [64].

This study showed that atezolizumab has acceptable drug-related toxicity and robust antitumor activity. An association between expression of PD-L1 in pretreatment samples and response to treatment with atezolizumab was described, with greater efficacy observed in tumor-infiltrating immune cell PD-L1 expression IHC score of 3. In the study, pretreatment specimens were scored based on their PD-L1 positivity. Specimens were scored IHC of 0, 1, 2, or 3 if <1%, 1–5%, 5–10%, or ≥10% of cells per area were PD-L1 positive, respectively. Tumor-infiltrating immune cell PD-L1 expression was associated with response to atezolizumab treatment, reaching statistical significance in NSCLC, p = 0.015. However, tumor cell PD-L1 expression was not associated with statistically significant response to atezolizumab across all tumors (p = 0.079) or NSCLC (p = 0.920) [64].

Eighty-three percent of patients with tumor-infiltrating immune cell IHC score of 3 responded to treatment with atezolizumab, with only 17% progressing, whereas in patients with IHC score of 2 (tumor-infiltrating immune cell), only 43% responded to treatment. However, the correlation was not observed between response to atezolizumab and expression of PD-L1 in tumor cell. In patients with tumor cell IHC 3,
Table 3. ongoing trials, from clinicaltrials.gov, involving immune checkpoint inhibitors as monotherapy or in combination with chemotherapy, targeted therapy, radiation, or other checkpoint inhibitors.

<table>
<thead>
<tr>
<th>Agent</th>
<th>NCT number</th>
<th>Trial name</th>
<th>Phase</th>
<th>Study population</th>
<th>Treatment arms</th>
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<tbody>
<tr>
<td>Anti-CTLA-4</td>
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<tr>
<td></td>
<td>NCT01998126</td>
<td>I Stage IV NSCLC with EGFR or ALK mutation</td>
<td></td>
<td>With targeted therapy (erlotinib or crizotinib)</td>
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<tr>
<td></td>
<td>NCT02381314</td>
<td>I Advanced melanoma, head/neck, NSCLC</td>
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<td>With MGA271</td>
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<td>Ipilimumab</td>
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<td></td>
<td>NCT02221739</td>
<td>II Metastatic NSCLC</td>
<td></td>
<td>With radiation</td>
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<tr>
<td></td>
<td>NCT01820754</td>
<td>II Clinical stage IB, stage IIA/IIIB or stage III NSCLC amenable to surgical resection</td>
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<td>Neoadjuvant therapy with chemotherapy (carbo/cisplatin + paclitaxel) + ipilimumab; followed by adjuvant ipilimumab</td>
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<td></td>
<td>NCT02279732</td>
<td>III Stage IV/recurrent squamous NSCLC</td>
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<td>With pembidoxel/carboplatin vs paclitaxel/carboplatin</td>
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<td>Tremelimumab</td>
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<td>NCT02040064</td>
<td>I Stage III/IV EGFR mutant disease that has progressed on EGFR inhibitors</td>
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<td>With gefitinib</td>
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<td>NCT02000947</td>
<td>Ib Advanced NSCLC</td>
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<td>With MEDI4736</td>
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<td>NCT02352948</td>
<td>III Stage IIIB/IV PD-L1 negative NSCLC, progressed after 2 lines of systemic treatment</td>
<td></td>
<td>Single agent tremelimumab vs investigators choice (vinorelbine, gemcitabine, erlotinib)</td>
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<td>Anti-PD-1</td>
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<td>Pembrolizumab</td>
<td>NCT01840579</td>
<td>Keynote-011 I Advanced NSCLC</td>
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<td>With cisplatin/pemetrexed or carboplatin/paclitaxel</td>
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<td></td>
<td>NCT0209449</td>
<td>I Advanced solid tumors</td>
<td></td>
<td>With AMO010</td>
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<tr>
<td></td>
<td>NCT02303990</td>
<td>I Advanced NSCLC failed anti-PD-1 therapy</td>
<td></td>
<td>With hypofractionated radiotherapy</td>
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<td>NCT02318771</td>
<td>I Head/neck, kidney, melanoma, NSCLC</td>
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<td>With radiation</td>
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<td>NCT02451930</td>
<td>I Stage IV metastatic NSCLC</td>
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<td>With necitumumab (LY301221)</td>
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<td></td>
<td>NCT02432963</td>
<td>I Recurrent NSCLC, breast carcinoma, ovarian carcinoma, pancreatic carcinoma, sarcoma</td>
<td></td>
<td>With p53MVA vaccine</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NCT02364609</td>
<td>I NSCLC with resistance to erlotinib</td>
<td></td>
<td>With afatinib</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NCT02443324</td>
<td>I Locally advanced and unresectable gastric/GEJ adenocarcinoma, NSCLC, or transitional cell carcinoma of the urothelium</td>
<td></td>
<td>With ramucirumab</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NCT02178722</td>
<td>I/II Advanced NSCLC</td>
<td></td>
<td>With INCBO24360</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NCT02039674</td>
<td>I/II Advanced or metastatic NSCLC</td>
<td></td>
<td>With chemotherapy (paclitaxel, carboplatin, bevacizumab, pemetrexed, erlotinib, gefitinib) or immunotherapy (ipilimumab)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NCT02407171</td>
<td>I/II Advanced NSCLC or metastatic melanoma</td>
<td></td>
<td>With stereotactic body radiation therapy (SBRT)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NCT02382406</td>
<td>I/II Advanced NSCLC</td>
<td></td>
<td>With carboplatin/nab-paclitaxel</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NCT02364609</td>
<td>I/II Stage IIA/IIIB/IV NSCLC with EGFR mutation</td>
<td></td>
<td>With afatinib</td>
<td></td>
</tr>
</tbody>
</table>
Table 3. Ongoing trials, from clinicaltrials.gov, involving immune checkpoint inhibitors as monotherapy or in combination with chemotherapy, targeted therapy, radiation, or other checkpoint inhibitors (cont.).

<table>
<thead>
<tr>
<th>Agent</th>
<th>NCT number</th>
<th>Trial name</th>
<th>Phase</th>
<th>Study population</th>
<th>Treatment arms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pembrolizumab (cont.)</td>
<td>NCT02422381</td>
<td>I/II</td>
<td>Advanced NSCLC</td>
<td>With gemcitabine</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NCT02444741</td>
<td>I/II</td>
<td>Stage IV metastatic NSCLC</td>
<td>With stereotactic body radiation therapy (SBRT) or wide-field radiation therapy</td>
<td></td>
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<tr>
<td></td>
<td>NCT02452424</td>
<td>I/II</td>
<td>Advanced NSCLC, melanoma, ovarian cancer, triple negative breast cancer, squamous cell cancer of head/neck, bladder cancer, pancreatic ductal adenocarcinoma, gastric cancer</td>
<td>With PLX3397</td>
<td></td>
</tr>
<tr>
<td>NCT02437136</td>
<td>Ib/Ii</td>
<td>Recurrent/metastatic NSCLC</td>
<td></td>
<td>With entinostat</td>
<td></td>
</tr>
<tr>
<td>NCT02085070</td>
<td>II</td>
<td>Metastatic NSCLC with brain metastasis</td>
<td></td>
<td>Single agent pembrolizumab</td>
<td></td>
</tr>
<tr>
<td>NCT02343952</td>
<td>II</td>
<td>Inoperable stage IIIA/IIIB NSCLC</td>
<td></td>
<td>Single agent pembrolizumab</td>
<td></td>
</tr>
<tr>
<td>NCT02316002</td>
<td>II</td>
<td>Oligometastatic NSCLC after progression on prior lines of treatment</td>
<td></td>
<td>Single agent pembrolizumab</td>
<td></td>
</tr>
<tr>
<td>NCT01905657</td>
<td>Keynote-010</td>
<td>II/III</td>
<td>Advanced NSCLC after progression on systemic therapy</td>
<td>a. High dose pembrolizumab</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>b. Low dose pembrolizumab</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>c. Docetaxel</td>
<td></td>
</tr>
<tr>
<td>NCT02142738</td>
<td>Keynote-024</td>
<td>III</td>
<td>Untreated stage IV NSCLC with +PD-L1</td>
<td>Single agent pembolizumab vs chemotherapy (paclitaxel, carboplatin, pemetrexed, cisplatin, gemcitabine)</td>
<td></td>
</tr>
<tr>
<td>NCT02220894</td>
<td>Keynote-042</td>
<td>III</td>
<td>PD-L1 + NSCLC</td>
<td>Single agent pembolizumab vs chemotherapy (carboplatin, paclitaxel, pemetrexed)</td>
<td></td>
</tr>
<tr>
<td>Nivolumab</td>
<td>NCT01454102</td>
<td>CheckMate 012</td>
<td>I</td>
<td>stage III/IV NSCLC</td>
<td>a. Single agent nivolumab</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>b. With erlotinib</td>
<td></td>
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<td></td>
<td>c. With ipilimumab</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>d. With bevacizumub</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>e. With gemcitabine/cisplatin</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>f. With pemetrexed/cisplatin</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>g. With carboplatin/paclitaxel</td>
<td></td>
</tr>
<tr>
<td>NCT02309177</td>
<td>I</td>
<td>Stage IIIB/IV NSCLC</td>
<td></td>
<td>With nab-paclitaxel and carboplatin</td>
<td></td>
</tr>
<tr>
<td>NCT01714739</td>
<td>I</td>
<td>Metastatic or unresectable solid tumors</td>
<td></td>
<td>With anti-KIR (lirilumab)</td>
<td></td>
</tr>
<tr>
<td>NCT02393625</td>
<td>I</td>
<td>ALK+ advanced NSCLC</td>
<td></td>
<td>With ceritinib</td>
<td></td>
</tr>
<tr>
<td>Agent</td>
<td>NCT number</td>
<td>Trial name</td>
<td>Phase</td>
<td>Study population</td>
<td>Treatment arms</td>
</tr>
<tr>
<td>-------------</td>
<td>-----------------------------</td>
<td>-------------------</td>
<td>--------</td>
<td>----------------------------------------------------------------------------------</td>
<td>-----------------------------------------------------</td>
</tr>
<tr>
<td>Nivolumab</td>
<td>NCT02335918</td>
<td></td>
<td>I/II</td>
<td>Advanced refractory solid tumor</td>
<td>With anti-CD27 (varlilumab)</td>
</tr>
<tr>
<td>(cont.)</td>
<td>NCT02327078</td>
<td></td>
<td>I/II</td>
<td>NSCLC, melanoma, colorectal neoplasm, squamous cell of head/neck, ovarian, B cell NHL or HL</td>
<td>With INCB24360</td>
</tr>
<tr>
<td></td>
<td>NCT02423954</td>
<td>Iv/II</td>
<td>Advanced solid tumors</td>
<td>a. With temsirolimus</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>b. With irinotecan</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>c. With irinotecan + capedatine</td>
</tr>
<tr>
<td></td>
<td>NCT02423343</td>
<td>Iv/II</td>
<td>Advanced or refractory NSCLC, hepatocellular carcinoma, or glioma</td>
<td>With transforming growth factor-beta receptor I kinase inhibitor (galunisertib)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NCT02323126</td>
<td>II</td>
<td>Advanced NSCLC with EGFR mutation or cMet positivity</td>
<td>With EGF816</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NCT01721759</td>
<td>CheckMate 063</td>
<td>II</td>
<td>Advanced or metastatic squamous cell NSCLC after failure of 2 prior therapies</td>
<td>Single agent nivolumab</td>
</tr>
<tr>
<td></td>
<td>NCT02259621</td>
<td>II</td>
<td>Resectable NSCLC</td>
<td>Neoadjuvant nivolumab</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NCT01642004</td>
<td>CheckMate 017</td>
<td>III</td>
<td>Advanced squamous NSCLC</td>
<td>Single agent nivolumab vs docetaxel</td>
</tr>
<tr>
<td></td>
<td>NCT01673867</td>
<td>CheckMate 057</td>
<td>III</td>
<td>Advanced nonsquamous</td>
<td>Single agent nivolumab vs docetaxel</td>
</tr>
<tr>
<td></td>
<td>NCT02041533</td>
<td>CheckMate 026</td>
<td>III</td>
<td>Stage IV or recurrent PD-L1+ NSCLC</td>
<td>Single agent nivolumab vs investigator's choice: gemcitabine, cisplatin, carboplatin, pacilaxel or pemetrexed</td>
</tr>
<tr>
<td></td>
<td>NCT02066636</td>
<td>CheckMate 153</td>
<td>IIIb/IV</td>
<td>Advanced NSCLC after progression on 1st line systemic therapy</td>
<td>Single agent nivolumab</td>
</tr>
<tr>
<td></td>
<td>NCT02409368</td>
<td>CheckMate 171</td>
<td>IIIb/IV</td>
<td>Advanced NSCLC after progression on at least 2 lines of systemic therapy</td>
<td>Single agent nivolumab</td>
</tr>
</tbody>
</table>

**Anti-PD-L1**

<table>
<thead>
<tr>
<th>Agent</th>
<th>NCT number</th>
<th>Trial name</th>
<th>Phase</th>
<th>Study population</th>
<th>Treatment arms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atezolizumab</td>
<td>NCT02400814</td>
<td>I</td>
<td>Stage IV NSCLC</td>
<td>With stereotactic ablative radiotherapy</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NCT02298153</td>
<td>I</td>
<td>Stage IIb/IV NSCLC previously treated with platinum-based chemotherapy</td>
<td>With INCB024360</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NCT01375842</td>
<td>I</td>
<td>Locally advanced or metastatic solid tumors or hematologic malignancies</td>
<td>Single agent atezolizumab</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NCT02174172</td>
<td>I</td>
<td>Advanced or metastatic NSCLC, renal cell carcinoma or melanoma</td>
<td>With ipilimumab in patients with NSCLC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NCT02013219</td>
<td>Ib</td>
<td>EGFR mutant, TKI naive advanced NSCLC</td>
<td>With Tarceva</td>
<td></td>
</tr>
</tbody>
</table>
## Table 3. ongoing trials, from clinicaltrials.gov, involving immune checkpoint inhibitors as monotherapy or in combination with chemotherapy, targeted therapy, radiation, or other checkpoint inhibitors (cont.).

<table>
<thead>
<tr>
<th>Agent</th>
<th>NCT number</th>
<th>Trial name</th>
<th>Phase</th>
<th>Study population</th>
<th>Treatment arms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atezolizumab</td>
<td>NCT02031458</td>
<td>Birch</td>
<td>II</td>
<td>PD-L1+ locally advanced or metastatic NSCLC</td>
<td>single agent atezolizumab</td>
</tr>
<tr>
<td>(cont.)</td>
<td>NCT01903993</td>
<td>Poplar</td>
<td>II</td>
<td>locally advanced NSCLC who have failed platinum therapy</td>
<td>single agent atezolizumab vs docetaxel</td>
</tr>
<tr>
<td></td>
<td>NCT01846416</td>
<td></td>
<td>II</td>
<td>PD-L1+ locally advanced or metastatic NSCLC</td>
<td>single agent atezolizumab</td>
</tr>
<tr>
<td></td>
<td>NCT02314481</td>
<td>Darwin II</td>
<td>II</td>
<td>Relapsed Stage III/IV NSCLC who are enrolled in the TRACERx study (NCT02183883)</td>
<td>a. if no actionable mutation =&gt; atezolizumab</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>b. BRAFV600 mutation =&gt; vemurafenib</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>c. HER2 amplification =&gt; trastuzumab emtansine</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>d. ALK/RET gene rearrangement =&gt; alectinib</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>e. Atezolizumab with interferon alfa-2b in patients with RCC or melanoma</td>
</tr>
<tr>
<td></td>
<td>NCT02409342</td>
<td>III</td>
<td></td>
<td>Chemo naive stage IV NSCLC</td>
<td>Single agent atezolizumab compared with (carboplatin or cisplatin + pemetrexed)</td>
</tr>
<tr>
<td></td>
<td>NCT02366143</td>
<td>III</td>
<td></td>
<td>Chemo naive stage IV nonsquamous NSCLC</td>
<td>With carboplatin/paclitaxel with and without bevacizumab</td>
</tr>
<tr>
<td></td>
<td>NCT02409355</td>
<td>III</td>
<td></td>
<td>Chemo naive stage IV squamous NSCLC</td>
<td>Atezolizumab compared with gemcitabine + cisplatin or carboplatin</td>
</tr>
<tr>
<td></td>
<td>NCT02367794</td>
<td>III</td>
<td></td>
<td>Chemo naive stage IV squamous NSCLC</td>
<td>With carboplatin + paclitaxel or carboplatin + nab-paclitaxel compared with carboplatin + nab- paclitaxel</td>
</tr>
<tr>
<td></td>
<td>NCT02367781</td>
<td>III</td>
<td></td>
<td>Stage IV nonsquamous NSCLC</td>
<td>With carboplatin + nab-paclitaxel compared with carboplatin + nab-paclitaxel</td>
</tr>
<tr>
<td></td>
<td>NCT02008227</td>
<td>Oak</td>
<td>III</td>
<td>Advanced or metastatic NSCLC after failure of platinum therapy</td>
<td>Atezolizumab compared with docetaxel</td>
</tr>
<tr>
<td>Durvalumab</td>
<td>NCT02088112</td>
<td>I</td>
<td></td>
<td>Locally advanced or metastatic NSCLC</td>
<td>With gefitinib</td>
</tr>
<tr>
<td></td>
<td>NCT02143466</td>
<td>I</td>
<td></td>
<td>EGFR mutant advanced NSCLC who have progressed with EGFR TKI</td>
<td>With AZD9291</td>
</tr>
<tr>
<td></td>
<td>NCT02000947</td>
<td>Ib</td>
<td></td>
<td>Advanced NSCLC</td>
<td>Combined with tremelimumab</td>
</tr>
<tr>
<td></td>
<td>NCT02403271</td>
<td>Ib/II</td>
<td></td>
<td>Relapsed or refractory solid tumors</td>
<td>With ibrutinib</td>
</tr>
<tr>
<td></td>
<td>NCT02087423</td>
<td>Atlantic</td>
<td>II</td>
<td>Locally advanced or metastatic NSCLC who have received at least 2 prior systemic treatment regimens</td>
<td>Single agent MEDI4736</td>
</tr>
<tr>
<td></td>
<td>NCT02273375</td>
<td>III</td>
<td></td>
<td>Patients with completely resected NSCLC</td>
<td>Adjuvant therapy compared with placebo</td>
</tr>
<tr>
<td></td>
<td>NCT02125461</td>
<td>III</td>
<td></td>
<td>Stage III unresectable NSCLC</td>
<td>Following concurrent chemoradiation compared with placebo</td>
</tr>
</tbody>
</table>
ORR was 46% whereas ORR in IHC 2 patients was 17%. Paired biopsy studies showed increase in PD-L1 expression on both tumor-infiltrating immune cells and tumor cells after treatment with atezolizumab. In patients with >30% reduction in tumor size, 5/6 (83%) patients had increase in PD-L1 on tumor-infiltrating immune cells and 3/6 (50%) patients had increase in PD-L1 in tumor cells. PD-L1 increase during treatment correlated with changes in tumor IFN-γ expression (Pearson correlation coefficient = 0.70) and activation of CD8 and TH1 responses [64].

The study also showed that expression of CTLA-4 in pretreatment tumors correlated strongly with response whereas fractalkine, a transmembrane protein and chemokine involved in adhesion and migration of leukocytes, identified in pretreatment tumors correlated with progression [67]. Elevated expression of IFN-γ as well as IFN-γ-inducible genes (IDO1 and CXCL9) in pretreatment tumors correlated with response in melanoma whereas these associations were weaker in NSCLC and renal cell carcinoma tumors. Evaluation of post-treatment biopsies showed lesions that regressed after treatment showed dense immune infiltrate and tumor cell necrosis. Sterilization of cancer cells occurred in some cases [64].

In tumors that failed to respond to treatment, little or no tumor-infiltrating immune cell infiltration were observed and minimal to no expression of PD-L1 were observed on intratumoral immune infiltrate. Biopsies obtained from tumors that progressed also showed presence of immune infiltrate present only around the outer edge of the tumor cell mass. Chip analysis showed that nonresponders failed to provide T cell activation and T-effector cell activity compared with those who responded to atezolizumab [68]. Foxp3, a transcription factor that plays at least a partial role in regulating Treg cell signature, was neither increased nor decreased in lesions that responded to treatment with atezolizumab [64,68].

Immune biomarkers were also examined in the blood, but did not significantly correlate with progression or response to treatment with atezolizumab. During cycle 1 of treatment with atezolizumab, increases in IL-18, ITAC (also known as CXCL11 or IP-9), proliferating CD8+ T cells (CD8+HLA-DR+Ki-67+) and IFN-γ were observed. Downtrend of IL-6 expression was observed by cycle 2, day 1 [64].

PD-L1 as a predictive biomarker was further examined in a Phase II study, FIR (NCT01846416), which evaluated atezolizumab in patients with stage IIIIB/IV NSCLC who were chemotherapy-naive (cohort 1), received ≥ 2 lines of therapy without brain metastasis (cohort 2), and received ≥ 2 lines of therapy with brain metastasis (cohort 3). PD-L1 expression was scored
Patients were scored as TC and IC 0, 1, 2, or 3 based on PD-L1 levels. Patients with TC3 or IC3 PD-L1 expression were found to be higher in squamous (21%) compared with nonsquamous (7%) disease. Adverse events were observed in 48% of patients. The most frequently reported drug-related adverse events were fatigue (14%), decreased appetite (9%) and nausea (8%). Grade ≥3 drug-related AEs were reported in 6% of patients, with 2% of patients having drug-related AEs that led to discontinuation of the study.

Combination of checkpoint inhibitors

Checkpoint inhibitors have provided remarkable clinical benefit for patients with advanced NSCLC, but they are not effective in all patients. Clinical trials are now evaluating the use of checkpoint inhibitors in combination to improve response rates. Combinations of CTLA-4 and PD-1 inhibitors are being studied in Phase I/II clinical trials in hopes that combination therapy will produce additive or synergistic activity. The use of checkpoint inhibitors are also being evaluated for use in combination with targeted therapy in patients who harbor activating EGFR mutations or as second line therapy in patients who have progressed on initial EGFR TKIs.

Interim Phase I results were reported for nivolumab and ipilimumab as first line in patients with advanced NSCLC (NCT01454102). The study included four cohorts of chemotherapy naive patients, both squamous and nonsquamous lung cancer who received nivolumab 3 mg/kg + 1 mg/kg ipilimumab followed by nivolumab 3 mg/kg IV every 2 weeks until disease progression or unacceptable toxicity. Forty-eight patients were evaluated at time of interim analysis. Overall ORR was 22% with stable disease rate of 33%. Of the seven squamous cell patients who received nivolumab 3 mg/kg + 1 mg/kg ipilimumab, there was 1 ongoing confirmed response (14%), 1 unconfirmed response (14%) and 2 patients with stable disease (29%). Of the 15 patients with nonsquamous NSCLC who received nivolumab 1 mg/kg + ipilimumab 3 mg/kg, there was 1 ongoing confirmed response (7%), 2 unconfirmed responses (13%) and 6 patients with stable disease (40%). Eight squamous cell NSCLC patients were treated with nivolumab 3 mg/kg + 1 mg/kg ipilimumab. Two patients had confirmed response (25%), none of whom were ongoing responders; three patients had unconfirmed response (38%), and stable disease was observed in four patients (50%). The last cohort of patients consisted of 16 patients with nonsquamous disease who received nivolumab 3 mg/kg + ipilimumab 1 mg/kg. Confirmed responses were observed in two patients (13%), both of whom were ongoing responders; four patients had unconfirmed response.
responses (25%), and stable disease was observed in three patients (19%). Treatment related adverse events were reported in 39 patients (85%). Grade 3–4 toxicities were reported in 22 patients (48%) which led to 16 patients to discontinue treatment. 2 patients were observed to have unconventional “immune related” responses. ORR was reported to not be correlated with PD-L1 status in the 29 tumor samples that was available for analysis at time of interim data. The results showed that combination of nivolumab and ipilimumab had acceptable toxicity and activity in both PD-L1+ and PD-L1- patients [75].

Another Phase I study examined the safety and tolerability of MEDI4736 with tremelimumab in patients with advanced tumors, including NSCLC, cervical, head and neck, colorectal, ovarian and renal cell carcinomas who were not eligible for, declined, or failed standard treatment (NCT01975831). The study design included dose escalation phase using a 3+3 design, with MEDI4736 administered at escalating doses starting at 0.3 mg/kg every 2 weeks; tremelimumab was administered at escalating doses starting at 3 mg/kg up to 10 mg/kg, every 4 weeks for the first six cycles and then every 12 weeks. Primary end points were safety/tolerability of MEDI4736 and tremelimumab when administered together. Preliminary data is still pending [76].

Combination of pembrolizumab plus ipilimumab as second line in stage IIIB/IV NSCLC is being studied in KEYNOTE-021 (NCT02039674). As of December 2014, 17 patients were enrolled to receive pembro 10 mg/kg + ipi 3 mg/kg, pembro 10 mg/kg + ipi 1 mg/kg, or pembro 2 mg/kg + ipi 1 mg/kg. Responses were seen at all dose groups without drug limiting toxicities or dose modifications. Eleven patients were on treatment for ≥26 weeks with 1 CR (9%) and 5 PRs (45%) [77].

**PD-1 Inhibition combined with targeted therapy**

Preclinical evidence suggests that PD-L1 tumor expression may be constitutively driven by EGFR signaling in EGFR mutant NSCLC. Treatment with anti-PD1 antibodies in preclinical EGFR mutant lung cancer models have demonstrated delayed tumor growth and increased survival [43,44].

Encouraging early activity has also been seen in the clinical setting when combining the anti-PD-1 antibody, nivolumab with erlotinib in patients with EGFR mutant NSCLC [78-40]. The Phase I study examined patients with Stage IIIB/IV EGFR mutant NSCLC who were chemotherapy naive or experienced progression after prior TKI therapy. Patients received nivolumab 3 mg/kg IV Q2W + erlotinib 150 mg PO daily until progression of disease or unacceptable toxicity. Interim analysis included 21 patients who received treatment for >10 months prior to analysis. Only one patient did not receive prior treatment with EGFR TKI. Of the 20 patients, who had acquired erlotinib resistance, 3 patients were observed to have partial responses, all ongoing. Duration of response were 6.1+, 16.3+ and 27.1+ weeks. Nine patients (45%) had stable disease with three patients (33%) having ongoing responses. One patient was observed to have unconventional ‘immune-related’ response that was ongoing at the time of interim analysis. Treatment related grade 3–4 adverse events were reported in four patients, which included increased AST or ALT, weight decrease and diarrhea. Two patients were discontinued from the study due to treatment related grade 3 AST increase and grade 2 nephritis. No cases of pneumonitis were reported at time of interim analysis [80].

**Checkpoint inhibitors & chemotherapy**

There are several ongoing studies examining the role of adding immune checkpoint inhibitors to standard chemotherapy regimens for treatment of NSCLC. From the 2015 ASCO meeting, preliminary data are available for pembrolizumab combined with chemotherapy and atezolizumab combined with chemotherapy in treatment naive patients.

Pembrolizumab is being investigated in combination with carboplatin AUC 6 + paclitaxel 200 mg/m² (cohort A: any histology) or carboplatin AUC 5 + pemetrexed 500 mg/m² (cohort C: nonsquamous without EGFR mutation or ALK translocation) in patients with treatment naive stage IIIB/IV NSCLC (NCT02039674). After four cycles, patients in cohort A received maintenance pembro and patients in cohort C received maintenance pembro + pemetrexed. As of interim analysis, Dec 2014, 44 patients were treated with preliminary ORR (confirmed and unconfirmed) of 30% in cohort A and 58% in cohort C. Grade 3–4 treatment related adverse events were observed in 15% of patients in cohort A and 38% in cohort C. One patient in cohort C discontinued the study due to grade 3 rash. This study showed promising ORR with pembrolizumab plus carboplatin and pemetrexed, which will be further evaluated in a larger cohort [81].

Atezolizumab was combined with chemotherapy in patients with previously untreated NSCLC (NCT01633970). The study combined atezolizumab with carboplatin + either paclitaxel (Arm C), pemetrexed (arm D), or weekly nab-paclitaxel (Arm E). At time of interim analysis on 29 September 2014, ORR was 67% (95% CI: 48–82%) across all arms, 60% (95% CI: 19–92%) in Arm C with 3 PRs, 75% (95% CI: 45–93%) in arm D with 9 PRs, and 62% (95% CI: 23–81% in Arm E.
33–83%) in arm E with 6 PRs and 2 CRs. The most common adverse events across all arms were nausea (Arms C and D, 50%; Arm E, 73%), fatigue (Arm C, 38%; Arm D, 36%; Arm E, 73%) and constipation (Arm C, 25%; Arm D, 71%, Arm E, 27%). The responses across all arms were independent of PD-L1 expression. Results showed that adding atezolizumab to standard first line chemotherapy produced promising clinical activity with acceptable drug related toxicities. Phase III studies evaluating this combination are still ongoing [82].

Combination of Nivolumab with platinum-based doublet chemotherapy as first line in NSCLC was evaluated in a Phase I multicohort study (NCT01454102). Preliminary data included 56 patients with advanced NSCLC who were assigned to four cohorts based on histology: nivolumab 10 mg/kg + gemcitabine/cisplatin for squamous cell, nivolumab 10 mg/kg + pemetrexed/cisplatin for nonsquamous cell, nivolumab 10 mg/kg + paclitaxel/carboplatin and nivolumab 5 mg/kg + paclitaxel/carboplatin for both squamous and nonsquamous cell lung cancer. ORR was 33–50% across all arms with no drug limiting toxicities observed during the first 6 weeks of treatment. Overall survival rates at one year were 59–87%. 45% of patients reported grade 3–4 treatment related adverse events (25–73% across all arms), including 4 patients with pneumonitis. This study is ongoing and currently demonstrates encouraging antitumor activity and 1-year overall survival [83].

**Future perspective**

PD-1/PD-L1 and CTLA-4 pathways appear to play dominant roles in T-cell immunosuppression. Blockade of these targets have been shown to produce antitumor activity. However, pre-existing immunity may be necessary, which is further amplified during treatment [64]. The tumor microenvironment has been shown to have four distinct subtypes: presence of both PD-L1 and TILs; presence of TILs without PD-L1; presence of PD-L1 with absence of TILs; absence of both PD-L1 and TILs [84,85]. Each subgroup likely has a different mechanism of immune resistance comprised of unique suppressive factors and inhibitory ligand-receptor interactions that need to be further characterized. On-treatment biopsies of nonresponders to PD-L1 therapy showed tumors with three patterns: an immunologic ignorance pattern displaying little or no tumor-infiltrating immune cell infiltration; nonfunctional immune response with presence of intratumoral immune infiltrate with minimal to no expression of PD-L1; excluded infiltrate response with immune filtrate residing solely on the outer edge of tumor cell mass [64]. Additional studies are needed to better understand immune regulatory pathways that may be involved in these nonresponders and identify treatment strategies to amplify immune response.

Identifying molecular markers to treatment response remains a challenge. Although some studies have shown that PD-L1 and CTLA-4 expression in tumor or immune microenvironment may correlate with response to therapy [53,64], other studies suggest that tumor PD-L1 status is not prognostic nor predictive of efficacy [51]. This discrepancy may be explained by heterogeneity of PD-L1 expression on biopsy samples leading to reporting inconsistencies, and technical difficulties with PD-1/PD-L1 analysis due to the harsh conditions required for antigen retrieval progress [3]. Table 2 summarizes the major biomarker driven trials described in this article, highlighting the different ways PD-L1 expression has been quantified. Further investigation is needed to develop improved diagnostic assays and identification of blood-based immune biomarkers.

The role of immunotherapy is also being explored in early stage lung cancer. Despite optimal treatment, recurrence rates remain high with 5-year survival of less than 50% [86–88]. Unpublished preliminary data have suggested that perhaps tumors at an earlier stage of disease may have higher PD-L1 levels compared with late stage disease [88]. Therefore, there may be a role for checkpoint inhibitors to improve recurrence rate and survival in early stage lung cancer. A Phase II trial is evaluating the role of nivolumab in the neoadjuvant setting in patients with resectable NSCLC (NCT0225962) and will further explore changes to cellular and molecular characteristics in the tumor microenvironment of early stage, potentially curable disease.

Novel multimodality treatment strategies are exploring combining checkpoint inhibitors with tumor vaccines (NY-ESO-1) and other new molecules. Table 3 summarizes current ongoing clinical trials involving immune checkpoint inhibitors. Nivolumab is being combined with lirlimumab, a monoclonal antibody against killer cell immunogloblin-like receptors (KIR) on NK cells, which has shown promising preclinical activity in lymphoma [89]. A Phase I/II study is exploring combination of nivolumab with varlilumab, an anti-CD27 antibody in patients with advanced solid tumors. Additional early phase clinical trials are evaluating immune check point inhibitors in combination with an antibody against B7-H3 (MGA271), with an inhibitor of Indoleamine 2,3-dioxygenase 1 (IDO1) which is a tryptophan-catabolizing enzyme overexpressed in cancer cells that suppresses T-cell response, and with pegylated recombinant human IL-10 (AM0010) [13,90–93].
Executive summary

**Immune checkpoint inhibition in lung cancer**

- Inhibition of immune checkpoints with anti-CTLA-4, PD-1 and PDL-1 agents have produced promising results demonstrating delayed tumor growth and increased survival. The durability of effect is most striking in those who respond.

**Anti-CTLA-4**

- CTLA-4 binds CD86 and suppresses activation and expansion of effector cells through regulation of Tregs. Inhibition of the CTLA-4 pathway with ipilimumab has improved immune-related progression free survival when given with standard chemotherapy, paclitaxel and carboplatin, compared with chemotherapy alone.

**Anti-PD-1/PD-L1**

- PD-1 is expressed by activated T cells and is a key immune checkpoint receptor that mediates immunosuppression. Inhibition of the interaction between PD-1 and PD-L1 has produced durable antitumor activity in NSCLC. Nivolumab has been FDA approved as second line treatment for metastatic squamous cell lung cancer and has now been shown to improve survival in patients with nonsquamous cell lung cancer as well. Phase I and II studies for anti-PD-L1 agents, atezolizumab (MPDL3280A) and durvalumab (MEDI4736), have shown these drugs have acceptable toxicity profile and have promising activity.

**Biomarkers**

- Tumor infiltrating immune cell PD-L1 expression and expression of CTLA-4 were associated with response to atezolizumab. However, there was not an association between tumor cell PD-L1 expression and response to atezolizumab. Fractalkine identified in pretreatment tumors was correlated with disease progression on atezolizumab treatment. Other smaller studies have shown increased efficiency of atezolizumab with high PD-L1 expression, IC or TC of 3.
- Higher response rate to pembrolizumab has been shown with tumor cell PD-L1 expression of ≥50%.
- There has been conflicting data on PD-L1 status and response to nivolumab. The CheckMate-017 study showed that PD-L1 status was not prognostic or predictive of efficacy, while CheckMate-057 showed that PD-L1 status correlated with response to treatment. These differences could be due to differences in the tumor types (i.e., smoking status) or speak to the need for better developed biomarker studies.

**Current limitations & future trends**

- Immune checkpoint inhibitors are being studied in the neoadjuvant setting for resectable disease to better characterize the tumor and immune microenvironment in early stage disease. Identification of molecular markers in the tumor, immune microenvironment and peripheral blood that correlate with treatment response or disease progression is needed. Further characterization of the immune profile in nonresponders and in those who progress on treatment will provide valuable information to help develop novel strategies to overcome resistance. New trials are now combining checkpoint inhibitors with chemotherapy, targeted therapy, radiation and other new antibodies in hopes of producing more durable response.

References

Papers of special note have been highlighted as: • of interest; •• of considerable interest

8. Shaw AT, Yap BY, Mino-Kenudson M et al. Clinical features and outcome of patients with non-small-cell lung...
19 Howard TA, Rochelle JM, Seldin MF, Cd28 and Cta-4, two related members of the Ig supergene family, are tightly linked on proximal mouse chromosome 1. Immunogenetics 33(1), 74–76 (1991).
21 Van Der Merwe PA, Bodian DL, Daenke S, Linsley P, Davis SJ. CD80 (B7-1) binds both CD28 and CTLA-4 with a low affinity and very fast kinetics. J.Exp. Med. 185(3), 393–403 (1997).
• This article demonstrated improved survival of ipilimumab in patients with metastatic melanoma, which contributed to its US FDA approval in 2011.


51 Spigel DR, Reckamp KL, Rizvi NA et al. A Phase III study (CheckMate 017) of nivolumab (NIVO; anti-programmed death-1 [PD-1]) vs docetaxel (DO) in previously treated advanced or metastatic squamous (SQ) cell non-small-cell lung cancer (NSCLC). ASCO Meeting Abstracts 33(Suppl. 15), 8009 (2015).


** Study showed survival advantage of nivolumab compared to docetaxel in squamous cell lung cancer and is also an important biomarker study that demonstrated PD-L1 status was not prognostic or predictive of efficacy.


** Important Phase III study that showed survival advantage of nivolumab in patients with nonsquamous cell lung cancer, but in contrast to CheckMate-017, PD-L1 expression was associated with improved efficacy.


** Article reported safety and efficacy of pembrolizumab and was also an important biomarker study showing that patients with PD-L1 proportion score of at least 50% had statistically significant better response.

60 Garon EB, Leighl NB, Rizvi NA et al. Safety and clinical activity of MK-3475 in previously treated patients (pts) with non-small-cell lung cancer (NSCLC). ASCO Meeting Abstracts 32(Suppl. 15), 8020 (2014).


** This article greatly contributed to the understanding of changes in tumor and tumor microenvironment after
treatment with atezolizumab and changes to inflammatory markers in peripheral blood.


67 CX3CR1 chemokine (C-X3-C motif) receptor 1 [Homo sapiens (human)]. www.ncbi.nlm.nih.gov/gene


79 Rizvi NA, Chow LQM, Borghaei H et al. Safety and response with nivolumab (anti-PD-1; BMS-936558, ONO-4538) plus erlotinib in patients (pts) with epidermal growth factor receptor mutant (EGFR MT) advanced NSCLC. ASCO Meeting Abstracts 32(Suppl. 15), 8022 (2014).


• Article described the tumor microenvironment in four distant subtypes and showed that mechanism of immune resistance was unique in each case.


90 Buchan SL, Manzo T, Flutter B et al. Ox40- and CD27-mediated costimulation synergizes with anti-PD-L1 blockade


92 Gibney GT, Hamid O, Gangadhar TC et al. Preliminary results from a phase 1/2 study of INCB024360 combined with ipilimumab (ipi) in patients (pts) with melanoma. ASCO Meeting Abstracts 32(Suppl. 15), 3010 (2014).


A pooled analysis of nivolumab for the treatment of advanced non-small-cell lung cancer and the role of PD-L1 as a predictive biomarker

Background: Recent studies with nivolumab (a monoclonal antibody against programmed cell death 1 [PD-1] receptor) have shown promise for non-small-cell lung cancer (NSCLC) treatment. Methods: To review available clinical trials data in order to assess nivolumab efficacy and the role of tumoral PDL-1 expression as a biomarker. Results: Nine eligible studies included 2102 patients. In the second line setting, nivolumab achieved a 1-year survival rate of 41%; and in the first line, a 1-year survival rate of 76%. For those with PD-L1 expression <1%, nivolumab showed a trend for improved survival compared with docetaxel. Conclusions: The available data reinforce nivolumab activity against NSCLC in first-line or subsequent lines. Although PD-L1 expression is related to greater response, PD-L1 negative patients had also some benefit.

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Keywords: lung cancer • meta-analysis • nivolumab

Most cancer cells emerge and develop through the acquisition and accumulation of several genetic alterations, mainly mutations [1,2]. These mutations can produce aberrant proteins that can serve as neoepitopes, which are recognized by the immune system [3]. The immune system is able to recognize and destroy tumor cells as well as pathogens. Nevertheless, one of the hallmarks of cancer is its ability to evade the immune system [4].

Programmed cell death 1 (PD-1) is a T-cell surface receptor that inhibits immune response when bound to its ligands (PD-L1 or PD-L2) present in normal human cells as well as in cancer cells. PD-1 is the target of immunotherapeutic agents such as nivolumab. Moreover, several other agents target PD-L1 [4–6]. Nivolumab is a fully human IgG4 antibody that enhances the immune response against cancer. Immune checkpoint inhibitors, such as nivolumab, have already been studied in many neoplasms and numerous other studies are ongoing [7,8].

Non-small-cell lung cancer (NSCLC) is among the main causes of cancer-related deaths worldwide [9]. Data from Surveillance, Epidemiology, and End Results (SEER) Program database of the American National Cancer Institute showed that 57% of the cases are diagnosed at stage IV (metastatic disease) and the 5-year survival rate in this group is only 1–4% [10]. In the last decade, several improvements in NSCLC therapy with the development of novel targeted cancer therapies, including monoclonal antibodies [11] and oral tyrosine kinase inhibitors have emerged [12]. Nevertheless, these agents are active only in a subset of patients whose tumors harbor specific genetic alterations [2]. There are few options to treat NSCLC, especially in the second-line setting [13].

The first studies with nivolumab have been conducted exactly in this setting and showed promising results [5,14–16]. The USFDA has already approved nivolumab for the second-line treatment of squamous-cell
and non-squamous cell NSCLC based upon results of two large Phase 3 trial [15,16].

Several studies regarding immune checkpoint inhibitors assessed the tumor PD-L1 expression as a predictive biomarker of such treatments with heterogeneous results [17]. Furthermore, PD-L1 expression assessment remains a controversial topic of debate in the scientific community. Taking under consideration all those paramount features, we conducted a pooled analysis to assess the efficacy and safety of nivolumab and the role of the tumor PD-L1 expression as a biomarker to predict tumor response and other parameters of clinical benefit.

Methods
Data sources
We searched for clinical trials in electronic databases (Pubmed, Lilacs and Cochrane Library) from 2000 to June 2015. MeSH terms included as follows: ‘non-small-cell lung cancer’, ‘anti PD-1’, ‘anti PD-L1’, ‘nivolumab’ and their synonyms. Second, we searched conference papers (American Society of Clinical Oncology (ASCO), European Society for Medical Oncology (ESMO) and International Association for the Study of Lung Cancer (IASLC)) in order to update included studies and search for additional eligible trials. Finally, we assessed references of included studies for potentially eligible studies.

Study selection
Study inclusion criteria were as follows: manuscript published in English; and prospective clinical trial that assessed efficacy and safety of nivolumab in the treatment of NSCLC, regardless of the number of previous treatments, tumor histology and study regimen.

Data extraction
We used The Cochrane Collaboration Guideline to assess study’s quality, collect data, evaluate the risk of bias and perform analyses. The authors analyzed the randomization process, the presence or absence of masking and the outcomes report in order to evaluate the risk of bias. Two independent reviewers assessed each eligible study [18]. All disagreements were resolved by an authors’ presential meeting and by a videoconference with a third reviewer. The data of feasible studies were extracted according to Cochrane’s protocol [19]. Study results were interpreted with caution regarding the risk of potential bias. All issues were assessed according to the findings published in the studies. Therefore, we did not ask for individual data.

Main outcomes & measures
The Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 guided image assessment for overall response rate (ORR) in all studies. Although immunotherapy may produce a heterogeneous response pattern that is not better evaluated by RECIST, all studies used this method because it is the most consolidated in the literature and it is easily audited or reproduced. The interval between each assessment was defined by each author; however, in the majority of cases, computed tomography or magnetic resonance imaging assessed the clinical activity at baseline and at 6, 12 and 16 weeks, and every 8 weeks thereafter. We considered either the investigator’s assessment or a central assessment.

Progression-free survival (PFS) was defined as the time, in months, from study inclusion until the first evidence of disease progression or death for any cause, whichever occurred first. In cases of short follow-up, we assessed the 24-week PFS rate. Overall survival (OS) was the time, in months, from study inclusion to death. Besides PFS, some trials did not evaluate the entire survival curve, and thus we considered the 1-year survival rate.

Studies that assessed tumor PD-L1 expression used immunohistochemistry with Dako North America antibodies (Antibody Dako 28–8). First trials established 5% as the optimal cut-off value but subsequent studies reported different results according to each cut-off value ranging from 1, 5 to 10%. In this article, we considered a cut-off value of 5%.

Nivolumab as well as other immune checkpoint inhibitors are generally well tolerated. Because of this, we did not include detailed adverse events analysis. Only highlights of the safety were cited in this manuscript.

Statistical analysis
Authors used Review Manager Version 5.3 in this assessment, developed and freely available from the Cochrane Collaboration. Pooled analysis of ORR, 24-week PFS rate and 1-year OS rate were assessed using descriptive statistics. Authors performed exploratory analysis according to the line of treatment and tumor histology. In this meta-analysis, we used the risk ratio (RR) for statistical efficacy analysis. Survival variables were evaluated using the hazard ratio (HR). We assessed heterogeneity using the $I^2$ statistic. A value less than 25% was minimal and from 25% to 50% was moderate. In these cases, the authors adopted the fixed model. Otherwise, $I^2$ higher than 50% indicated substantial heterogeneity and the random model was used. p-values <0.05 were considered statistically significant.

Results
Search result
An initial search identified 82 studies. Only three
Nivolumab for the treatment of advanced NSCLC & the role of PD-L1 as a predictive biomarker

Research Article

complied with the inclusion criteria [14,15,20]. Six additional studies were identified after the conference papers review [7,16,21–25]. A total of 2102 patients were included in the nine eligible studies. Figure 1 illustrates the search process.

Eight studies assessed nivolumab in previously treated patients; two of them were randomized Phase 3 trials comparing nivolumab with docetaxel in the second-line setting. In the first-line setting, there was only one trial with different regimens in each arm [21,23–25]. One of these arms included ipilimumab in combination with nivolumab. Ipilimumab is a monoclonal antibody against another receptor that inhibits T-cells death. This different pathway may confuse the results, particularly regarding PD-L1 as a biomarker. However, we included this study arm because of the paucity of first-line setting studies. Table 1 summarizes the characteristics of the included studies. The number of patients treated with nivolumab among all included trials was 1643 (459 patients were treated with docetaxel). Table 2 describes the main characteristics of patients treated with nivolumab.

Efficacy & safety: first-line setting

A large Phase I trial assessing the safety and efficacy of nivolumab in the first-line setting is ongoing. It has eight arms; in this analysis, we assessed four of them separately. Despite a substantial heterogeneity among treatment regimens, nivolumab showed activity in the first-line setting. Among 146 patients included in these reports, 29% achieved tumor response and the 1-year survival rate was 76%.

Overall, nivolumab was well tolerated in the first-line setting. Reports described 40% of adverse events of any grade; however, they were manageable, especially if detected early. There were few cases of treatment interruption and treatment-related three deaths were reported. Although immune-mediated adverse events, mainly pneumonitis, are a worrying side effect, they occurred in a minority (9% of patients; 5% were grade 3 or 4).

Figure 1. PRISMA statement of the search result.
<table>
<thead>
<tr>
<th>Study</th>
<th>First author</th>
<th>Year</th>
<th>Phase</th>
<th>Population</th>
<th>Dose</th>
<th>n</th>
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<td>2014</td>
<td>I</td>
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<td>1 mg/kg q2w 3 mg/kg q2w 10 mg/kg q2w</td>
<td>33 37 59</td>
<td>Dako</td>
<td>Tumor</td>
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<td>RECIST 1.0</td>
<td>[20]</td>
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<td>SQ NSCLC after failure</td>
<td>3 mg/kg q2w Docetaxel 75 mg/m² q3w</td>
<td>135 137</td>
<td>Dako</td>
<td>Tumor</td>
<td>5%</td>
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<tr>
<td>CheckMate 057</td>
<td>Paz-Ares</td>
<td>2015</td>
<td>III</td>
<td>non-SQ NSCLC after failure</td>
<td>3 mg/kg q2w Docetaxel 75 mg/m² q3w</td>
<td>292 290</td>
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<td>Tumor</td>
<td>5%</td>
<td>RECIST 1.1</td>
<td>[16]</td>
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<tr>
<td>Bauer</td>
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<td>NA</td>
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<td>12 15 15 14</td>
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<td>[23]</td>
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<td>24 25</td>
<td>Dako</td>
<td>Tumor</td>
<td>5%</td>
<td>RECIST 1.1</td>
<td>[21]</td>
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**Efficacy & safety: second-line setting & beyond**

In the second-line setting and beyond, the ORR was 17% among 1204 patients assessed. Analyzing by histology, squamous cell NSCLC achieved higher response (21% among 451 patients) than nonsquamous cell NSCLC (15% among 752 patients; p = 0.01). Interestingly, the 1-year survival rate was lower among patients with squamous cell NSCLC (32 vs 48%; p < 0.01). For the entire population, four studies with 673 patients were analyzed showing a 1-year survival rate of 41%.

Nivolumab was well tolerated in second- or third-line: in five studies with 1488 patients, 25% experienced grade 3 or 4 adverse events. Pneumonitis occurred in 29 (2%) patients 11 (0.7%) of which were severe (grade 3 or 4).

**Nivolumab vs docetaxel in the second-line setting**

Two Phase 3 trials addressed this issue. In their meta-analysis, nivolumab reached a statistically significant higher ORR (RR: 1.74; 95% CI: 1.25–2.43; Figure 2A), as well as a better prognosis (PFS HR: 0.82; 95% CI: 0.71–0.95; Figure 2B and OS HR: 0.69; 95% CI: 0.58–0.81; Figure 2C) compared with docetaxel in the second-line setting.

**PD-L1 expression as a predictive biomarker**

Six studies with 576 patients assessed the PD-L1 expression as a biomarker of clinical benefit. Among 237 PD-L1 ‘positive’ patients (>5% of expression), the ORR was 27%. Among 339 PD-L1 ‘negative’ patients (<5% of expression), the ORR was 13%. Heterogeneity
was minimal and this difference was statistically significant (RR: 2.04; 95% CI: 1.37–3.04; Figure 3A). PD-L1 positive patients also had a non-statistically significant higher 24-week PFS rate (RR: 0.93; 95% CI: 0.79–1.11; Figure 3B) and 1-year survival rate (RR: 0.90; 95% CI: 0.65–1.26; Figure 3C).

Nivolumab was numerically superior to docetaxel even among patients with a PD-L1 expression <1%. ORR was 13 versus 12% (RR: 1.04; 95% CI: 0.58–1.87) and there was a trend towards better OS (HR: 0.78; 95% CI: 0.60–1.01). The role of PD-L1 as a predictive biomarker for overall survival was more relevant for non-squamous tumors (patients with PD-L1 <1% HR: 0.90; 95% CI: 0.66–1.24) than for squamous tumors (patients with PD-L1 < 1% HR: 0.58; 95% CI: 0.37–0.92).

Risk of bias assessment
The authors considered that the high proportion of undefined bias in the study’s patient randomization was because many trials did not have a control arm. The high risk of bias in the blinding was not harmful to analysis results, and it is possible to allow this because many studies were not Phase 3 trials and had to assess the safety of these novel agents. In general, another type of bias (outcomes report) was considered low.

Discussion
Nivolumab showed activity in the treatment of NSCLC regardless of tumor histology and number of previous treatments. The agent was slightly more active among patients with squamous-cell histology. It has been hypothesized that in squamous cell NSCLC, tumor cells have a high mutation burden, and consequently, they can produce more aberrant proteins as well as neoepitopes recognized by the immune system [3]. On the other hand, patients with squamous-cell NSCLC had a worse 1-year survival rate. We presume that tumor biology and availability of few postprogression treatment options may explain this finding.

Although in the first-line setting the number of patients was small and the heterogeneity of regimens was substantial, we feel these results may stimulate the development of Phase 2 or 3 trials assessing this issue. The design of these novel trials presents a major challenge. First, there are many regimens as well as targeted therapies to combine and/or to compare. Second, it is important to consider that nivolumab as well as other immune checkpoint inhibitors has a specific pattern of antitumor activity and may not be the best option for all patients in this setting. Furthermore, tumor response criteria using the RECIST v1.1 may not be the most appropriate marker of clinical activity and may lead to an under-estimation of the immunotherapeutic drug efficacy [3]. Studies described unusual tumor responses compared with those currently observed for cytotoxic agents as follows: lesions that grow first and then start to reduce in size, lesions that reduce while new lesions develop, or lesions stable for a long period. This promoted the development of new response assessment criteria [26]. However, this criteria was not commonly used in clinical trials. Therefore, patient inclusion criteria and study end points should be chosen carefully.

Despite the aforementioned constraints regarding tumor response with nivolumab, we argue that the results of this analysis in the second-line and beyond are an important contribution to the current state of the art. The FDA currently approved nivolumab for the treatment of patients with either squamous or non-squamous NSCLC who were previously treated with chemotherapy or targeted therapy. Of note, previous studies in patients with advanced melanoma with longer follow up showed tumor response sustained for 4

<table>
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<th>Table 2. Baseline characteristics of the patients.</th>
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n: Number of patients, ECOG: Eastern Cooperative Oncologic Group Performance Status Criteria.
long time period [27]. Nivolumab also showed activity and survival superior to docetaxel regardless of tumor histology. In the current meta-analysis, nivolumab had a statistically significant better PFS compared with docetaxel. We believe these results enhance the evidence regarding nivolumab superiority over docetaxel for the second-line treatment of NSCLC regardless of histology.

In general, nivolumab was well tolerated and adverse events were manageable. The high suspicion for immune related adverse events and a quick response to them reduces the number of patients with severe adverse events. The PD-L1 expression was assessed as a predictive biomarker for immune checkpoint inhibitors because of its relationship with the agents’ target. However, there is currently no standard approach to assess this biomarker in routine clinical practice and the optimal cut-off value remains to be defined. Nevertheless, all studies used immunohistochemistry with the same antibody and the same assessment criteria. The PD-L1 expression was not a reliable predictive biomarker, despite the higher ORR observed among PD-L1 positive patients. Among patients with a PD-L1 expression < 1% nivolumab also had considerable activity for non-squamous histology and was superior to docetaxel. For nonsquamous tumors, PD-L1 seems to be more reliable. We conclude that the study of other biomarkers is warranted to improve prediction of clinical activity in this setting.

**Conclusion**

Nivolumab shows activity in advanced NSCLC in both first- and second-line settings and it was superior to docetaxel with respect to ORR, PFS and OS in both squamous cell and nonsquamous cell NSCLC. Tumor PD-L1 overexpression was related to higher ORR; however, nivolumab was also active among patients with PD-L1 expression < 1% and nonsquamous histology.

**Future perspective**

Further studies are warranted to identify a reliable biomarker and to assess the role of nivolumab in the first-line setting.

An extended follow-up may emphasize the long-term benefits of nivolumab and may help to identify predictive features for patients with response sustained for a long time.

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**Figure 2. Clinical outcomes regarding nivolumab versus docetaxel.**

(A) Overall response rate – nivolumab vs docetaxel, (B) progression-free survival – nivolumab vs docetaxel, (C) overall survival – nivolumab vs docetaxel.
Nivolumab for the treatment of advanced NSCLC & the role of PD-L1 as a predictive biomarker

Author contributions

Dr de Mello had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: de Mello. Acquisition, analysis, or interpretation of data: Aguiar Jr, de Mello, Mountzios, Santoro, Oliveira, Castelo-Branco, Lopes Jr. Drafting of the manuscript: Aguiar Jr, Lopes Jr, Tadokoro, Mountzios, de Mello. Critical revision of the manuscript for important intellectual content: Statistical analysis: Administrative, technical, or material support: Study supervision: de Mello.

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Financial & competing interests disclosure

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Figure 3. Clinical outcomes according to the PD-L1 status. (A) Overall response rate by PD-L1 expression status, (B) 24-weeks progression-free survival rate by PD-L1 expression status, (C) 1-year survival rate by PD-L1 expression status.
Executive summary

- Few treatment options are available for squamous non-small-cell lung cancer despite recent advances, especially in the second-line setting.
- Nivolumab has been studied in several neoplasms such as lung cancer. The antibody is currently being assessed in the first-line and subsequent lines of treatment. The aim of this study is to assess the efficacy of nivolumab and the role of PD-L1 as a predictive biomarker.

Methods

- A pooled analysis of all available data regarding nivolumab for the treatment of advanced lung cancer.
- Nine trials with 2102 patients were included.

Results

- Nivolumab has shown activity in the first-line and subsequent lines of treatment.
- In second-line setting, nivolumab achieved an overall response rate (ORR) of 17% (five studies, 1204 patients) and a 1-year survival rate of 41% (four studies, 673 patients).
- PD-L1 expression was a predictive biomarker for tumor response.
- Patients with PD-L1 < 1% and squamous histology had also significant activity with nivolumab.

Discussion

- Nivolumab is active in the treatment of advanced lung cancer, but further studies should find a more reliable predictive biomarker and better evaluate nivolumab in the first-line of the treatment.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

References

Nivolumab for the treatment of advanced NSCLC & the role of PD-L1 as a predictive biomarker


Immune checkpoint inhibitors have been identified as breakthrough treatment in melanoma given its dramatic response to PD-1/PD-L1 blockade. This is likely to extend to many other cancers as hundreds of clinical trials are being conducted or proposed using this exciting modality of therapy in a variety of malignancies. While immune checkpoint inhibitors have been extensively studied in melanoma and more recently in lung cancer, little is known regarding immune checkpoint blockade in other cancers. This review will focus on the tumor immune microenvironment, the expression of PD-1/PD-L1 and the effect of immune modulation using PD-1 or PD-L1 inhibitors in patients with head and neck, prostate, urothelial, renal, breast, gastrointestinal and lung cancers.

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Keywords: atezolizumab • immune checkpoint blockage • immune checkpoint inhibitors • immunotherapy • nivolumab • PD-1 • PD-L1 • pembrolizumab • solid tumors • tumor infiltrating lymphocytes

Beyond melanoma: inhibiting the PD-1/PD-L1 pathway in solid tumors

The discovery of immune checkpoints represents a new era in cancer treatment. Recently, it became evident that tumors can hijack the immune tolerance mechanism by overexpressing these immune checkpoints placing a brake on the activation of immune system [1]. Inhibiting these immune checkpoints or their ligands can lift the break on the immune system leading to a powerful immune response against tumor cells. Not surprisingly, immune checkpoints were first investigated in melanoma given the well-known immunogenic nature of this malignancy [2]. The CTLA-4 antibody ipilimumab was the first immune checkpoint inhibitor that showed activity in melanoma treatment [2] followed by PD-1 inhibitors [3,4]. Since then many studies have been conducted to expand the indication of immune checkpoints beyond melanoma. This review will focus on these studies targeting PD-1/PD-L1 pathway in cancers other than melanoma.

The Programmed Death Pathway (PD-1) is an inhibitory receptor that belongs to the B7-receptor family and binds to its ligands PD-L1 (also known as B7-H1) and PD-L2. When T cells are activated they start to express PD-1 which interacts with PD-L1 on tumor cells leading to T-cell deactivation and apoptosis, therefore, downmodulating the immune response against tumors [5,6]. PD-1 is expressed on a variety of tumor infiltrating lymphocytes (TILs) such as activated T- and B-lymphocytes, natural killer (NK) cells and myeloid cells while PD-L1 is expressed not only on tumor cells but also on stroma, antigen presenting cells and tumor-associated macrophages [5,6]. The downregulation of the T-cell response is thought to allow the unchecked growth of tumor cells and also represents one of the many tumor immune escape mechanisms that regulate the immune response against cancer cells [1,7]. More recently, a new stratification of tumors was proposed...
based on PD-L1 status and presence or absence of the TILs [8]. Based on this classification, tumors with PDL-1 and TILs positive were called ‘immune resistant’ since the expression of PD-L1 was thought to be driven by TIL-induced INF-γ, while tumors with both PD-L1 and TILs negative were called ‘immune ignorant’. On the other hand, the induction of PD-L1 in PD-L1 + TILs – tumors was defined as ‘intrinsic induction’ that may be related to oncogenic induction of PD-L1 rather than TILs driven. Finally, the presence of TILs in PD-L1- tumors was blamed on other suppressors in a type called ‘tolerant tumours’. This classification may help to better understand the biology of the PD-1/PD-L1 pathway in order to choose an appropriate immunotherapy [9]. Although the expression of PD-L1 was reported to correlate with poor survival in many malignancies including NSCLC, GU and GI cancers [10–12] this correlation remains unclear given the retrospective nature of this data. Considerably less is known about PD-L2. Compared to the expression of PD-L1 in most cancers, constitutive PD-L2 expression is low but can increase in response to Th2 cytokines leading to the suppression of CTL reactivity [13]. The overexpression of PD-L2, however, has been found in a variety of cancers including primary mediastinal B-cell and Hodgkin lymphoma [14] but its correlation to survival remains unclear.

Squamous cell carcinoma of the head & neck

Much research has elicited the risk factors of alcohol, tobacco use and the human papillomavirus (HPV) in head and neck cancer, suggesting a role of chronic inflammation that results in immune system escape and tumor growth [15]. Despite this, little is known regarding the lack of immune response in squamous cell carcinoma of the head and neck (SCCHN). The expression of PD-L1 is higher in SCCHN patients with HPV infection compared with those without (70 vs 29%), possibly due to the inflammatory reaction that is driven by HPV infection [15]. Targeting the PD-1/PD-L1 pathway is an active area of investigation, primarily resulting from abstracts at international conferences, although multiple trials are ongoing to define the role of these agents in the management of SCCHN. The KEYNOTE-012 multi-cohort Phase Ib study of the anti-PD-1 antibody pembrolizumab included patients with advanced SCCHN. In 2014, an analysis of 60 selected PD-L1-positive patients from this group with recurrent/metastatic SCCHN was presented [16]. PD-L1 expression in stroma or ≥1% tumor cells was required for inclusion. These patients received pembrolizumab 10 mg/kg every 2 weeks. The overall response rate (ORR) was 20% and was similar in HPV positive and negative patients. 58% of patients experienced a drug-related adverse effect (AE). Subsequently, at the 2015 ASCO Annual Meeting, results from a different SCCHN cohort from the KEYNOTE-012 trial including PD-L1 unselected patients were presented [17]. 132 patients with recurrent/metastatic SCCHN were treated with a fixed dose of pembrolizumab 200 mg IV every 3 weeks. ORR was 18.2% and 47% of patients had a drug-related AE. Furthermore, early studies of the anti-PD-L1 antibody durvalumab (formerly MEDI4736) reported tumor shrinkage in treated patients with recurrent/metastatic SCCHN [18]. CTLA-4 blockade is under investigation as single agent therapy and in combination with anti-PD-1 therapy for recurrent/metastatic SCCHN.

Glioblastoma multiforme

Glioblastoma multiforme (GBM) is another aggressive malignancy with little known about its relationship to the immune system. Recent data from the 2015 ASCO Annual Meeting evaluated TILs infiltration as well as PD-1 and PD-L1 expression in GBM tumor samples from 117 patients [19]. Dense immune cell infiltration was not found, but sparse infiltration of CD8+ cells was found in 44% of tumors with spare PD-1 expression on TILs seen in 29% of tumors. PD-L1 expression on tumor cells was observed in 38% of tumor samples (defined as ≥5%). No correlation was found between PD-1 or PD-L1 and TILs density or patient outcome. Interestingly, 22% of tumors were PD-L1+ initially but lost PD-L1 expression at the time of recurrence. In the same meeting, preliminary results were presented on cohort 1 of the Phase I CHECKMATE-143 trial [20]. Twenty patients with recurrent GBM were treated with single agent nivolumab 3 mg/kg every 2 weeks or combination nivolumab 1 mg/kg + ipilimumab 3 mg/kg every 3 weeks followed by nivolumab 3 mg/kg every 2 weeks. All patients were in their first recurrence, had no prior bevacizumab and a Karnofsky Performance Score >70 with primary endpoint of safety/tolerability. No grade 3–4 AEs were seen in the nivolumab alone group, whereas nine out of ten patients in the combination group had a grade 3–4 AE (most commonly lipase elevation, fatigue and diarrhea). Overall survival at 6 months in both groups was 75%. Pembrolizumab is currently under investigation in this patient population (NCT02337491 and NCT02337686).

Urothelial carcinoma

Advanced urothelial carcinomas (transitional cell carcinoma of the bladder, renal pelvis, ureter and urethra) are currently treated with cisplatin-based chemotherapy, with significant challenges in this patient population due to advanced age, comorbid conditions and poor tolerance. For nonresponders, there is limited
benefit of second and subsequent lines of cytotoxic chemotherapy, highlighting the need for effective therapy. Urothelial cancers have been shown to have a high rate of somatic mutations and possible neo-antigens, suggesting the potential success of targeted- and immuno-therapies [21]. In one study of 65 urothelial cancer cases, PD-L1 was found to be expressed in all tumor samples and tumors with expression of PD-L1 ≥ 12.2% were found to have higher grade pathological features and worse prognosis [10]. Accordingly, targeting this pathway is an active area of research, with promising early results. Two multicohort Phase I trials of checkpoint inhibitor monotherapy have included patients with recurrent/metastatic urothelial cancer. In 2014, data from the Phase I urothelial cancer expansion cohort evaluating the safety and anti-tumor activity of atezolizumab (formally known as MPDL3280A, an anti-PD-L1) was reported [22]. All patients had immunohistochemical (IHC) analysis of tumor-infiltrating immune cells for PD-L1. Patients had either high PD-L1 expression (IHC 2/3) or low-expression (IHC 0/1). Initially, only PD-L1-positive (IHC 2/3) patients were included, but the cohort was ultimately expanded to include all patients regardless of IHC result. 67 patients who received atezolizumab at a dose of 15 mg/kg or 1200 mg IV every 3 weeks for up to 1 year were evaluated for safety and efficacy. Over 93% of these patients had received prior platinum-based therapy and many had poor prognostic features. The ORR for patients with a minimum of 6 weeks of follow-up was 43% for IHC 2/3 tumors including a 7% complete response (CR). For patients with IHC 0/1 tumors, the ORR was 11%. Half of the patients reported a drug-related AE. Based on this, the FDA granted atezolizumab breakthrough status in urothelial bladder cancer. Updated results from this trial were presented at the 2015 ASCO Annual Meeting [23]. Out of the 85 patients with at least 12 weeks of follow-up for efficacy evaluation, 46 were IHC 2/3, 38 were IHC 0/1 and 1 had unknown IHC status. The ORR for the IHC 2/3 group was 46% including six CR. The ORR for the IHC 0/1 group was 16% and all were partial response (PR). Pembrolizumab has also been tested in patients with recurrent/metastatic urothelial carcinoma as part of the multicohort Phase I KEYNOTE-012 trial. Initial results of the urothelial cohort were reported in 2014 and updated at the 2015 ASCO Annual Meeting. 33 patients with ≥ 1% of tumor cells expressing PD-L1 by IHC received 10 mg/kg pembrolizumab IV every 2 weeks until complete response, progression or unacceptable toxicity. 76% of the patients had prior therapy and 66% had visceral or osseous metastases. The ORR for evaluable patients was 24% including three CR. 64% of patients treated with pembrolizumab had decrease in the size of target lesions. The rate of grade 3 or 4 toxicity was 12% [24].

Renal cell carcinoma
With the recent US FDA approval of nivolumab for metastatic renal cell carcinoma (RCC), there is much excitement about the role of PD-1 inhibition in this malignancy. PD-L1 expression of ≥1% has been shown in roughly 50% of RCC and has been correlated to a more advanced stage, higher grade and worse overall survival [25,26]. Interestingly, one study showed similar expression levels of PD-L2 [27]. While early studies were primarily in clear cell histology, this high level of expression seems to exist regardless of histology type, with an even higher level of expression (89%) in patients with sarcomatoid histology [28]. PD-1 inhibition was first studied in 33 patients with pretreated metastatic RCC who received nivolumab at a dose of 0.1–10 mg/m2 every 2 weeks as a part of the Phase 1 nivolumab study. This cohort of RCC had an ORR of 27% [29]. This led to Phase II [30] and III [31] trials which recently led to nivolumab’s FDA approval in RCC after progression on anti-angiogenic therapy. This is based on demonstrating an overall survival (OS) of 25 months compared with 19.6 months with everolimus (HS: 0.73, p = 0.002). OS was not statistically different based on PD-L1 expression suggesting PD-L1 may not be a useful biomarker in RCC and further studies are needed to explain this lack of correlation. ORR was also improved compared with everolimus (25 vs 5%, p < 0.001), including four CRs in the nivolumab group. Grade 3 or higher AEs were reported in fewer patients receiving nivolumab compared with everolimus (19 vs 37%) and quality of life scores were improved in the nivolumab group [31]. PD-L1 inhibition has also been shown to have a promising activity in renal cell carcinoma. In the multicenter Phase I trial, BMS-936559 (PD-L1 inhibitor) was given to a cohort of patients with metastatic renal cell carcinoma at a dose of 10 mg/kg every 2 weeks. ORR was 12%, with 41% having stable disease [32]. Grade 3 or higher AEs were 5% and PFS was 53% at 24 weeks. Recently at the 2015 ASCO Annual Meeting, combination therapy of nivolumab and ipilimumab in pretreated patients with metastatic RCC was presented. ORR was found to be 39% in the group treated with nivolumab 1 mg/kg and ipilimumab 3 mg/kg compared with 29% when treated with nivolumab 3 mg/kg and ipilimumab 1 mg/kg. 43 patients reported grade 3 or higher AEs [33]. Pembrolizumab is currently being studied in the neoadjuvant setting in patients with resectable disease (NCT02212730).
Prostate cancer
The FDA-approval of sipuleucel-T, an autologous dendritic cell-based cancer vaccine against prosthetic acid phosphatase (PAP), in 2010 was the first evidence of immunotherapy improving survival in this patient population [34]. Since then, other forms of immune therapy including anti-CTLA-4 therapy and PD-1 therapy have been tested without the success seen in other disease sites. While there were early studies showing activity of ipilimumab in castration-resistant disease [35], a Phase III trial was unable to show a survival benefit [36]. In one of the few studies evaluating the PD-1/PD-L1 pathway, no tumor samples of prostate cancer showed expression of PD-L1 by IHC [29]. A cohort of men with castration-resistant prostate cancer (CRPC) were included in the initial Phase I study with nivolumab (a PD-1 inhibitor) [29], however no objective responses were observed in the 17 patients. Optimizing immunotherapy for prostate cancer remains an area of active investigation. With the lack of response to date, various combinations with chemotherapy, varying patient populations and predictive biomarkers are currently being investigated.

Colorectal cancer (CRC) & anal cancer
The ‘immunoscore’ was first described in colorectal cancer and found to outweigh the TNM staging system in predicting prognosis [37–40]. This ‘immunoscore’ is calculated based on the density and the location (in the center of the tumor and the invasive margins) of CD3+ T cells and CD8+ T cells [41] and is currently being validated globally. In addition, there is a mounting evidence to suggest that the host develops spontaneous humoral and cellular immune responses against CRC tumor antigens [42]. On the other hand, CRC may have a direct immunosuppressive effect at the molecular and cellular level with suppression of cell-mediated immunity. Strong PD-L1 (defined as ≥5 cells per 0.0625 mm²) expression was observed in 30% of CRC patients and was found to correlate with infiltration by CD8+ lymphocytes which did not express PD-1 [43]. However, targeting the PD-L1/PD-1 pathway in CRC resulted in disappointing outcomes. No response was reported in 19 CRC patients treated on the first-in-human Phase I trial of nivolumab [29]. During 3 years follow-up, one patient achieved an objective response on an intermittent dosing regimen following discontinuation of therapy [44]. In another study using a PD-L1 inhibitor, none of the 18 CRC patients treated on this dose escalation Phase I study had a clinical response [32]. More recently, a subset of CRC tumors with mismatch repair deficiency (MRD) was reported to show a response rate of 40% to PD-1 inhibition compared with 0% in mismatch repair-proficient (MRP) colorectal cancers [45]. This was due to the high somatic mutation loads that mismatch repair-deficient tumors have leading to an immune response against their coded antigens. Further studies are investigating the efficacy of PD-1 inhibition in a larger cohort of CRC patients with MRD (NCT02563002). In addition, we are investigating the combination of anti-PD-1 and chemoradiation therapy (CRT) in patients with rectal cancer in the neoadjuvant setting, based on preliminary data showing that CRT can lead to an increase in TILs [46] which could be further enhanced and activated by blocking the PD-1/PD-L1 pathway (NCT02586610).

In a recent abstract at the European Cancer Congress 2015, Ott et al.; presented results from KEYNOTE-028, an ongoing Phase Ib trial of pembrolizumab in patients with previously treated anal cancer [47]. All patients were required to have PD-L1 positivity of at least 1% by IHC. Forty-seven patients were screened, with 38% having PD-L1 expression. Out of the 25 patients enrolled, ORR was 20% to pembrolizumab given in a dose of 10 mg/kg every 2 weeks up to 2 years or until progression or unacceptable toxicity. One complete response and 40% stable disease were observed. Grade 3 or more AEs were reported in 8% of the patients.

Pancreatic cancer
It had been speculated that pancreatic cancer may not be responsive to immunotherapy due to the unique pancreatic cancer tumor microenvironment (PCTM). TILs rarely infiltrate the PCTM, although their density in the PCTM had been reported to correlate with better clinical outcomes [48]. On the other hand, the PCTM is associated with a massive infiltration of suppressive immune cells that contribute to a worse prognosis [49]. More importantly, pancreatic cancer is associated with a dense desmoplastic stroma that forms a barrier for immune cells and interacts with cancer cells leading to tumor progression and invasion [50]. The PD-1/PD-L1 pathway have been targeted in pancreatic cancer given the fact that pancreatic cancer tumors that express PD-L1 were found to have worse prognosis [12, 51]. However, no clinical response was reported in 14 pancreatic cancer patients who received BMS-936559 (a fully human IgG monoclonal antibody targeting PD-L1) [32]. At the ASCO 2015 Annual Meeting, results from 24 patients with pancreatic cancer treated with another PD-L1 antibody (durvalumab) were presented. While the majority of patients progressed through PD-L1 inhibition, 2/24 (8%) showed a partial response [18]. The ongoing KEYNOTE-28 study is investigating pembrolizumab (anti-PD-1) in a
variety of malignancies including pancreatic cancer. In addition, we are currently testing whether the addition of CRT to anti-PD-1 could overcome the resistance of pancreatic cancer to anti-PD-1 therapy by creating an inflammatory microenvironment that could lead to a better immune response against pancreatic cancer (NCT 02305186).

**Cholangiocarcinoma**

The knowledge regarding the immune profile of cholangiocarcinoma is very limited due to the low incidence of the disease and its poor prognosis. Cholangiocarcinoma patients with intraepithelial tumor-infiltrating CD4+, CD8+ TILs had a significantly longer overall survival compared with patients with low TILs [52]. Recently, Rosenberg et al., demonstrated a complete response in a patient with metastatic cholangiocarcinoma after adoptive transfer of TILs containing about 25% mutation-specific poly-functional T(H)1 cells [53]. Not only were the effector T cells found to correlate with survival in cholangiocarcinoma but also the expression of the immune checkpoints on tumor cells. Expression of PD-1 and PD-L1 was found to be upregulated in intrahepatic cholangiocarcinoma (ICC) tissues compared with the cancer adjacent tissues and significantly correlated with both poor tumor differentiation and advanced pathological stage and was inversely correlated with CD8+ TILs [54]. Therefore, the PD-1/PD-L1 pathway may be linked to malignant potential of ICC and may contribute to tumor immune evasion by promoting CD8+ TILs apoptosis. Thus, this pathway may indeed be a potential therapeutic target in the treatment of cholangiocarcinoma.

**Hepatocellular carcinoma (HCC)**

The immune system has been shown to play an important role in the course of HCC. HCC is considered an inflammation-associated cancer since hepatitis B and C virus infection are known to be the major risk factors. In addition, few immune cell subsets have been associated with poor outcome in HCC including tumor-infiltrating CD4+ regulatory T cells [55,56] and myeloid-derived suppressor cells (MDSC) [57]. The overexpression of PD-L1 had been also associated with poor prognosis in HCC [58]. This observation had led to a Phase I/II study using nivolumab in patients with advanced HCC who failed sorafenib but have a Child-Pugh score of 7 or less. The preliminary result of this study was recently presented at the 2015 ASCO annual meeting indicating a promising effect of nivolumab in HCC with an overall response rate of 23% including two complete responses in all disease subsets whether they had hepatitis B, C or no infection [59].

**Gastric & gastro-esophageal junction cancers**

Similar to other malignancies in the GI tract, there is mounting evidence to suggest that the unique immune microenvironment of gastric and gastro-esophageal junction (GEJ) cancers includes an abundance of suppressive immune cells compared with activating immune cells [60,61]. The density of PD-1 positive CD4+ and CD8+ T cells in gastric cancer samples were found to be higher than normal healthy controls and a higher density correlated to worse disease progression, poor tumor differentiation and lymph node metastases [60]. PD-1 and PD-L1 expression has correlated not only with worse overall survival, but also with malignant transformation [60,62–64]. Recently at the 2015 ASCO Annual Meeting, a study of 105 tumor samples from patients with metastatic gastric cancer found that PD-1 positive TILs were present in 26% of samples and disease-free survival at 3 years was worse in patients with PD-1 positive TILs compared with tumors that did not (36 vs 65%, p = 0.022) [65].

The KEYNOTE-012 trial included 162 patients with gastroesophageal cancer. All were assessed by IHC for PD-L1 expression, with 40% having at least 1% expression or more. In this single arm Phase Ib study, 10 mg/kg pembrolizumab (anti-PD-1) was given every 2 weeks for up to 24 weeks and showed an RR of 22% and 6-month PFS and OS of 24 and 69%, respectively [66]. There was a nonsignificant trend toward an association between higher levels of PD-L1 expression and ORR and OS (p = 0.102 and 0.124, respectively). Subsequently, at the 2015 ASCO Annual Meeting, data correlating four prespecified multigene immune response signatures (IFN-γ, TCR signaling, Expanded immune and Denovo) from 33 patients with advanced gastric cancer treated with pembrolizumab on KEYNOTE-012 was presented [67]. Analysis suggested a low gene signature score correlated with a lack of response. This may be of significant benefit to predict patients who will respond to PD-L1 therapy and those who will not. In another study, one gastric cancer patient was treated with atezolizumab (anti-PD-L1) every 3 weeks, as a part of Phase I study. This patient’s tumor sample was 3+ for PD-L1 by IHC and clinically showed partial response at 6 weeks with eventual progression at week 51 [68].

**Breast cancer**

Out of the major subtypes of breast cancer, triple-negative breast cancer (TNBC) and HER2 positive are most consistently associated with the presence of TILs and are currently thought to be the most amenable to anti-PD-1 and other checkpoint inhibitor-based therapies. Clinical studies suggest that the presence of TILs
may predict effectiveness of therapy in HER2+ and triple-negative breast cancer \([69,70]\). In contrast, for luminal A, estrogen receptor positive, breast cancer, CD8 infiltrates were correlated with increased risk of death in a 12,000 tumor series. Clinical response to immunotherapy in ER-positive breast cancer has been disappointing to date in early phase studies \([71]\). There are several explanations for poor response to immunotherapy in ER-positive breast cancers, including the relatively scarcity of neo-antigens, the relative frequency of myeloid-derived suppressor cells (MDSC’s), the unfavorable cytokine milieu, and the relatively lower levels of lymphocytic infiltrates compared with other solid tumors \([72,73]\). There are biologic reasons to expect TNBC and HER2+ breast cancer to be amenable to PD-1-based therapies. The rationale includes strong immunogenicity of the HER2 protein, the elevated mutational loads of these subtypes, the purported high levels of neo-antigen formation and the reliance on nonestrogenic signaling pathways. Recently, there are hints that relative TIL infiltrates may have predictive potential for response to adjuvant HER2-based therapies \([74]\). Despite identification of PD-L1 on HER2+ breast cancer \([75]\) and despite encouraging preclinical data \([76]\), there have been no published prospective clinical trials of checkpoint inhibitors in HER2+ disease; an anti-PD-1 trial is currently accruing (NCT02129556). In TNBC, there is a little more clinical data to suggest a role for anti-PD-1-based therapy. For example, a study of 116 breast cancers found PD-1 expression on the surface of 50% of TILs, while PD-L1 expression was detected in 45% of breast cancer tumor cells. Concurrency between PD-1 on TILs and PD-L1 on tumors was observed 29% of the time \([77]\). Not surprisingly, PD-1+ TILs were more frequent in TNBC samples (70%) than in ER+ and HER2+ subtypes \((25–44\%, \ p < 0.001)\) \([77]\). Likewise, PD-L1 was observed on the triple-negative cancer cells more than ER+ and HER2+ types \((59 \text{ vs } 33\%, \ p = 0.017)\). RNA sequencing from The Cancer Genome Atlas found that TNBC mRNA expression of PD-L1 was nearly twice as high as other subtypes \((p < 0.001)\) \([78]\). Likewise, a tissue microarray found PD-L1 expression in 19% of TNBC samples \([78]\). Finally, a comparative genomic hybridization study of 326 tumors found PD-L1 and PD-L2 high level amplification in 29% of TNBC \([79]\).

Completed clinical trials of PD-1 targeting agents include a pembrolizumab study in TNBC reported at the 2014 San Antonio Breast Cancer meeting by Nanda et al. \([80]\). The Nanda Phase Ib study enrolled 32 heavily pretreated patients with recurrent or metastatic TNBC and with any level of PD-L1 expression in their tumor. Based on their assay methods, only 58% of all screened patients had PD-L1+ tumors. In the study, 10 mg/kg of pembrolizumab was given every 2 weeks, and treatment remained ongoing for patients whose disease was not clearly progressing as assessed by RECIST v1.1 every 8 weeks. Treatment was tolerable with only 16% of patients experiencing grade 3–5 toxicity, but one treatment-related death from disseminated intravascular coagulation was reported. The overall RR was 19% with one (4%) complete response, four (15%) partial responses and seven patients (26%) with stable disease. In this population, the median time to response was 18 weeks (range, 7–32). A second trial of a PD-L1 inhibitor was presented at AACR 2015 by Leisha Emens \([81]\). In that Phase I trial, atezolizumab was given to 12 TNBC patients with metastatic disease. Grade 3–4 toxicity occurred in one patient with renal insufficiency to date and there were no treatment-related deaths. The overall RR was 33% in the nine patients evaluable for efficacy, with one CR and two PRs. All responses were seen within the first 6 weeks of treatment. Another 17 PD-1 studies in breast cancer are currently listed at clinicaltrials.gov.

Non-small-cell lung cancer (NSCLC)

With the recent FDA approvals of immune checkpoint inhibitors for adenocarcinoma and squamous lung cancers, interest in the tumor microenvironment and immune interaction has increased dramatically over the past several years. In one study, 56% of tumor samples with lung adenocarcinoma and squamous cell histology showed PD-L1 positivity ≥1% and correlated with worse prognosis \([11]\). Efforts to engage this interaction for the treatment of lung cancer have progressed at a feverish pace, particularly with the development of checkpoint inhibitors.

Single agent immunotherapy

**PD-1 Inhibition**

The initial report of the CHECKMATE 001 study of nivolumab was promising because it demonstrated single agent immunotherapy activity in NSCLC and suggested that the responses were prolonged in some patients \([29]\). CHECKMATE 001 ultimately enrolled 129 previously treated NSCLC patients (54% had received at least three lines of prior therapy) \([82]\). AEs were observed in 41.1% of patients; six patients experienced grade 3 or greater AEs, four of which were due to pneumonitis (one of which was grade 5) \([82]\). The ORR was 17.1% with median duration of response of (DOR) 17.0 months (range 1.4 to 36.8 months) \([82]\). Responses were observed in both squamous (9 out of 54 patients) and nonsquamous histology (11 out of 74 patients) and across all dose levels studied \([82]\). PD-L1 status was not predictive of responses, though archived tumor tissue was not available for all patients and pretreatment biop-
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In this study, pembrolizumab, was the subject of the large Phase I trial KEYNOTE 001 to examine the safety and efficacy of this drug in both previously treated and treatment-naive NSCLC patients [83]. Among the total study population (n = 495, 394 previously treated and 101 treatment-naive) the ORR was 19.4% (18.0% among prior treated and 24.8% in treatment naive) with stable disease (SD) in 21.8% [83]. Patients with a PD-L1 proportion score ≥ 50%, defined as a proportion of cells with ≥1% over all cells (n = 73), had an RR of 45.2% and median PFS of 6.3 months for all patients, 6.1 months for previously treated patients and 12.5 months for treatment-naive patients [83]. Among patients with PD-L1 proportion scores less than 1% or between 1 and 49%, the median PFS and OS were similar in both groups, but comparatively less than those with a score ≥ 50% [83]. As with nivolumab, there were low rates of treatment-related AEs (fewer than 10% with grade ≥ 3). The KEYNOTE 001 trial demonstrated that response rates, PFS, and OS were increased in those patients with high PD-L1 staining; however there does not seem to be a scientific basis for choosing a cut point of ≥ 50%, a seemingly arbitrary selection. Nevertheless, pembrolizumab is now FDA approved for patients with PD-L1-positive NSCLC whose disease has progressed after other treatments. After the first report of responses to nivolumab in NSCLC, multiple Phase II and Phase III clinical trials were opened to further investigate checkpoint inhibitors in NSCLC. Final results were first reported from trials that focused on patients with squamous cell NSCLC (SC-NSCLC), a histology that had little progress compared with nonsquamous NSCLC (NS-NSCLC) in recent years. CHECKMATE 063 was a single arm, Phase II study of nivolumab 3 mg/kg in 6117 patients, 65% of who had received at least three prior lines of therapy [84]. The ORR was 14.5% and the SD rate was 26% among a population of patients with best response (CR or PR) of 4% to the most recent line of therapy [84]. Thirteen of 17 patients with a CR or PR had ongoing responses at the time of the analysis, and the median OS in this group had not been reached [84]. CHECKMATE 017 was a randomized, Phase III trial that compared nivolumab to docetaxel for second-line treatment in 272 patients with SC-NSCLC [85]. Treatment with nivolumab led to a 3.2-month improvement in median OS compared with docetaxel (9.2 vs. 6.0 months; p <0.001). ORR was also improved with nivolumab compared with docetaxel (20 vs 9%; p = 0.008) [85]. Based on the CHECKMATE 063 and CHECKMATE 017 studies, the FDA approved nivolumab for the treatment of second-line SC-NSCLC. A similar randomized Phase III trial of nivolumab versus docetaxel, CHECKMATE 057, was conducted for patients with NS-NSCLC [86]. This trial randomized 582 patients after progression following first-line platinum doublet chemotherapy to nivolumab (n = 292) or docetaxel (n = 290). As in the CHECKMATE 017 trial, nivolumab showed a significant improvement in median OS (12.2 vs 9.4 months; p = 0.001) and ORR (19 vs 12%; p = 0.02) over docetaxel. The investigators reported improved safety and tolerability of nivolumab over docetaxel with all grade and high grade (grade 3–4) toxicities higher with docetaxel when compared with nivolumab (88 vs 69% and 54 vs 10%, respectively). Based on this study, nivolumab was also FDA approved for patients with NS-NSCLC. Most recently, the KEYNOTE 010 trial, a randomized Phase II/III trial of two doses of pembrolizumab versus docetaxel chemotherapy, also showed an OS improvement with pembrolizumab [87]. The CHECKMATE 017, 057 and KEYNOTE 010 trials have convincingly demonstrated that anti-PD-1 therapy improves response rates and survival for patients with NSCLC and has a better toxicity profile than docetaxel chemotherapy in second-line therapy.

PD-L1 inhibition

PD-L1 inhibition has shown efficacy in advanced SC- and NS-NSCLC as well as second-line therapy. BMS-936559, a fully human IgG monoclonal antibody toward PD-L1, was studied in 207 patients with a variety of solid tumors [32]. Among the 75 patients with NSCLC, five patients experienced a PR with three patients experiencing a PR at 24 weeks [32]. Another PD-L1 antibody, atezolizumab (formerly MPDL3280A) was studied in a Phase I dose escalation study that enrolled 88 patients with NSCLC and compared responses according to PD-L1 expression in tumor cells and tumor infiltrating immune cells [88]. Patients with the highest PD-L1 expression in tumor or immune cells had an ORR of 45 versus 14% for patients with low or absent PD-L1 expression [88]. Interim results of a larger Phase II study, POPLAR, that compared atezolizumab to docetaxel after prior platinum doublet therapy were reported at the ASCO Annual Meeting in 2015 [89]. OS was improved in the group with high PD-L1 expression with the median not reached in those receiving atezolizumab compared with patients who received docetaxel (median 11.1 months) [89]. Interim OS was not significantly different for the entire intent to treat (ITT) analysis that contained a total
of 288 patients in both cohorts, though this may change with longer follow-up [89]. Atezolizumab is also the subject of an ongoing study in chemotherapy-naïve NSCLC patients (NCT01846416). Durvalumab is another anti-PD-L1 IgG1 antibody that has been studied in patients with advanced NSCLC after first-line therapy and in novel combinations. An update to the ongoing Phase I/II study of durvalumab in advanced NSCLC was presented at the ASCO Annual Meeting in June 2015 [90]. Among the 198 patients treated, 149 were evaluable for response with ≥ 24 weeks of follow-up [90]. The ORR was 14% among all evaluable patients, though it was higher in PD-L1-positive patients (23%) and in SCC compared with NS-NSCLC (21 vs 10%, respectively) [90]. Avelumab (also known as MSB0010718C) is another anti-PD-L1 IgG1 antibody that is currently being evaluated in NSCLC. In a Phase I expansion cohort

![Figure 1. PD-1/PD-L1 inhibition & response rate. (A) PD-1 inhibition based on cancer, drug and overall response rate. (B) PD-L1 inhibition based on cancer, drug and overall response rate. (C) Combination therapy based on cancer, drug and overall response rate.

**Microsatellite-instability-high tumors only.**

CRC: Colorectal carcinoma; HCC: Hepatocellular carcinoma; NSCLC: Non-small-cell lung carcinoma; ORR: Overall response rate; OS: Overall survival; RCC: Renal cell carcinoma; SCLC: Small-cell lung carcinoma; uro: Urothelial.
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treating 184 NSCLC patients, there was a 13.5% RR and 50.5% disease control rate. Only 12.5% of patients had grade 3 or higher treatment-related adverse events [9]. A Phase III randomized trial of avelumab versus docetaxel is ongoing (JAVELIN Lung 200, NCT02395172).

**Figure 2. Response rate based on PD-L1 expression.** (A) NSCLC, all histologies. (B) Urothelial cancer. (C) Other cancers.

*≥50% tumor cells PD-L1+; **1–49% tumor cells PD-L1+.

NSCLC: Non-small-cell lung carcinoma, all histologies; ORR: Overall response rate; OS: Overall survival; PD-L1+: ≥1% expression; PD-L1-: ≤1% expression; RCC: Renal cell carcinoma; SCCHN: Squamous cell carcinoma of head/neck; SCLC: Small-cell lung carcinoma; Uro: Urothelial.
Combination therapy

PD-1 inhibition in combination with chemotherapy or targeted therapy

In addition to monotherapy trials in previously treated patients, checkpoint inhibitors have been studied in the first-line setting and in combination with other checkpoint inhibitors or chemotherapy agents. The CHECKMATE 012 trial (NCT01454102) is an ongoing Phase I trial with multiple arms (currently A through S) evaluating nivolumab as monotherapy (Arm F) or in combination with various agents. Results from nivolumab in combination with platinum doublet chemotherapy have been presented on multiple occasions [92,93]. Fifty-six patients treated on these arms of the study received nivolumab concurrently with cisplatin/gemcitabine (A), cisplatin/pemetrexed (B) or carboplatin/paclitaxel (C and C5). Treatment was well tolerated and there were no dose-limiting toxicities (DLT) in the first 6 weeks. At the time of recent report, the median OS in each of these arms was (A) 50.5 weeks, (B) 83.4 weeks, (C) 64.9 weeks and (C5) not reached [93]. For patients treated on Arm F with single-agent nivolumab, 11 of 52 (21%) had an objective response, three with complete response [94]. Median OS was 98.3 weeks and 1-year OS rate presented was 74%. Notably, 15% of patients in this arm had EGFR mutations. Additional cohorts of patients divided by histology are being enrolled for nivolumab first-line treatment. Another combination trial, KEYNOTE 021 (NCT02039674), is a Phase I/II trial evaluating pembrolizumab in combination with platinum doublet chemotherapy, targeted therapy (erlotinib or gefitinib) or immunotherapy (ipilimumab). Results from 44 patients treated on cohorts A and C, carboplatin/paclitaxel and carboplatin/pemetrexed respectively, were recently reported [95]. As with the CHECKMATE 012 study, these results also demonstrated safety with these PD-1 plus platinum doublet combinations as only one patient experienced a DLT. ORR was 30 and 56% in Arms A and C, respectively. Cohort G, an open-label Phase II portion of this trial is currently accruing and randomizing patients to carboplatin/pemetrexed with or without pembrolizumab. Pembrolizumab in combination with carboplatin and nab-paclitaxel is currently being investigated in a Phase I/II clinical (NCT02382406).

PD-L1 inhibition in combination with chemotherapy or targeted therapy

Fewer studies have investigated PD-L1 inhibition in combination compared with PD-1 inhibition. Atezolizumab has been studied in first-line treatment of NSCLC in combination with platinum doublet chemotherapy, and as with the CHECKMATE 012 and KEYNOTE 021 studies, the combination anti-PD-L1 therapy with chemotherapy was safe and tolerable [100]. Durvalumab is also the subject of a multicohort study combined with an EGFR inhibitor (osimertinib) with or without selumetinib (MEK inhibitor) in EGFR mutant NSCLC (NCT02143466) and in combination with the anti-CTLA-4 antibody tremelimumab (NCT02000947).

Small-cell lung cancer

In the SCLC arm of the CHECKMATE 032 trial [97], 90 patients with progressive SCLC after platinum doublet chemotherapy were treated with nivolumab alone (n = 40) or in combination with ipilimumab (n = 50) [97]. Grade 3–4 treatment-related toxicity in the nivolumab and ipilimumab combination arms was 34%, with only 2.1% developing grade 3–4 pneumonitis. Response rates were 17 and 18% with median duration of response not reached and 6.9 months in the nivolumab and combination cohorts, respectively. Three patients on this study developed limbic encephalitis. Although the numbers of cases of this autoimmune toxicity are small, this may be a new safety signal specific to patients with SCLC.

Conclusion

The use of anti-PD-1 and anti-PD-L1 antibodies has generated significant excitement in the oncology community with numerous ongoing studies in variety
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of cancers (summarized in Tables 1 & 2 & Figures 1 & 2). Without doubt, the benefits of PD-1/PD-L1 inhibition outweigh their adverse effects, particularly compared with traditional chemotherapy. While prolonged and durable responses were achieved using immune checkpoints inhibitors, only a relatively small number of patients experience such clinical responses. Although there are emerging data that some malignancies have very high response rates, for example, Merkel cell carcinoma (RR of 80%) [101], significant efforts are still needed for why certain malignancies respond to immunotherapy while others seem untouched by its use.

Moreover, the predictive value of PD-L1 as a biomarker that may determine which patients are more likely to respond to PD-1/PD-L1 blockade remains debatable given the fact that a subset of patients with PD-L1-negative tumors still responds to PD1/PD-L1 blockade. The variability of response based on PD-L1 expression could be related to many factors including: using different cut-off to define PD-L1 positivity (1% vs 5 or 50%); the variation of the staining method (different antibodies, manual vs automatic count, pathologist training); the variation in cellular distribution of the staining (membrane vs cytoplasmic); the location of PD-L1 expression (tumor cells vs immune cells); tissue acquisition (fresh vs archived); the heterogeneity of tumors and the dynamic changes in the tumor microenvironment. Indeed, substantial efforts are underway to address this debate with FDA calling for a validated method of staining and pharmaceutical companies teaming up to launch prospective trials to answer these questions [102]. However, the expression of PD-L1 may be useful in making clinical decision as shown recently in melanoma. Particularly, in melanoma patients with

Table 1. Expression of PD-L1 and its correlation with pathological features and survival.

<table>
<thead>
<tr>
<th>Cancer type</th>
<th>PD-L1 expression on tumor cells</th>
<th>PD-1 expression on TILs reported</th>
<th>Associated pathologic features</th>
<th>Survival</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head/neck</td>
<td>70% (HPV+)§</td>
<td>+</td>
<td>↑ IFN-γ</td>
<td>NR</td>
<td>[15]</td>
</tr>
<tr>
<td></td>
<td>29% (HPV-)§</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genitourinary</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urothelial</td>
<td>21%‡</td>
<td>+</td>
<td>↑ grade and tumor size</td>
<td>↓</td>
<td>[10]</td>
</tr>
<tr>
<td>RCC</td>
<td>24–50%‡</td>
<td>+</td>
<td>↑ grade, ↑ pathological stage</td>
<td>↓</td>
<td>[25,26,31]</td>
</tr>
<tr>
<td>Prostate</td>
<td>0%</td>
<td>-</td>
<td>N/A</td>
<td>N/A</td>
<td>[29]</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colorectal</td>
<td>30%§</td>
<td>-</td>
<td>↓ CD8+ infiltration, ↑IFN-γ</td>
<td>↑ if MMR proficient</td>
<td>[43]</td>
</tr>
<tr>
<td>Pancreatic</td>
<td>39%§</td>
<td>-</td>
<td>↓ CD8+ &amp; CD4+ infiltration</td>
<td>↓</td>
<td>[12,51]</td>
</tr>
<tr>
<td>CC</td>
<td>5.45†</td>
<td>+</td>
<td>↑ grade, ↑ pathological stage, ↓ CD8+ infiltration</td>
<td>↓</td>
<td>[54]</td>
</tr>
<tr>
<td>HCC</td>
<td>33% (defined as ≥10%)</td>
<td>-</td>
<td>↑ recurrence rate postresection, ↑ FoxP3+ infiltration</td>
<td>↓</td>
<td>[58]</td>
</tr>
<tr>
<td>Gastric</td>
<td>40%§</td>
<td>+</td>
<td>↑ grade, ↑ pathological stage, ↓ CD8+ infiltration, ↑ metastases</td>
<td>↓</td>
<td>[62,65]</td>
</tr>
<tr>
<td>Esophageal</td>
<td>52% (defined as ≥10%)</td>
<td>-</td>
<td>↑ grade, ↑ pathological stage, ↑ metastases</td>
<td>↓</td>
<td>[64]</td>
</tr>
<tr>
<td>Breast</td>
<td>45%§ (59% in TNBC; 100% in metaplastic)</td>
<td>+</td>
<td>↑ CD8+ infiltration</td>
<td>↓</td>
<td>[75,77,78]</td>
</tr>
<tr>
<td>Lung</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NSCLC, adeno</td>
<td>65%§</td>
<td>-</td>
<td>↑ in adeno (compared with SCC)</td>
<td>↓</td>
<td>[11,85]</td>
</tr>
<tr>
<td>NSCLC, squamous</td>
<td>44%§</td>
<td>+</td>
<td>NR</td>
<td>↓</td>
<td>[11]</td>
</tr>
</tbody>
</table>

Staining intensity score.

≥1%, ≥5%.

CC: Cholangiocarcinoma; HCC: Hepatocellular carcinoma; HPV: Human papilloma virus; MMR: Mismatch repair; N/A: Not applicable; NR: Not reported; NSCLC: Non-small-cell lung cancer; RCC: Renal cell carcinoma; SSC: Squamous cell carcinoma; TILs: Tumor infiltrating lymphocytes; TNBC: Triple-negative breast cancer.
Table 2. Clinical outcomes of PD-1/PD-L1 inhibition in solid tumors other than melanoma.

<table>
<thead>
<tr>
<th>Cancer type</th>
<th>Drug</th>
<th>Patient population</th>
<th>ORR</th>
<th>DCR</th>
<th>Drug-related AE (grade ≥3)</th>
<th>Survival benefit</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head/neck</td>
<td>Pembrolizumab</td>
<td>PD-L1+ ≥1%</td>
<td>20%</td>
<td>NR</td>
<td>17%</td>
<td>NR</td>
<td>[16]</td>
</tr>
<tr>
<td>Genitourinary</td>
<td>Atezolizumab</td>
<td>Unselected</td>
<td>43% (PD-L1 ≥5%)</td>
<td>NR</td>
<td>3.2%</td>
<td>NR</td>
<td>[22]</td>
</tr>
<tr>
<td></td>
<td>Pembrolizumab</td>
<td>PD-L1+ ≥1%</td>
<td>24%</td>
<td>NR</td>
<td>12%</td>
<td>NR</td>
<td>[24]</td>
</tr>
<tr>
<td>RCC</td>
<td>Nivolumab</td>
<td>Unselected</td>
<td>25%</td>
<td>59%</td>
<td>19%</td>
<td>↑ OS by 5.4 mos vs everolimus (HR 0.73, p = 0.002)</td>
<td>[31]</td>
</tr>
<tr>
<td></td>
<td>BMS-936559</td>
<td>Unselected</td>
<td>12%</td>
<td>53%</td>
<td>5%</td>
<td>53% PFS at 24 weeks</td>
<td>[32]</td>
</tr>
<tr>
<td></td>
<td>Nivolumab + Ipilimumab</td>
<td>Unselected</td>
<td>39%</td>
<td>78%</td>
<td>43%</td>
<td>NR</td>
<td>[33]</td>
</tr>
<tr>
<td>Prostate</td>
<td>Nivolumab</td>
<td>Unselected</td>
<td>0%</td>
<td>0%</td>
<td>14%</td>
<td>N/A</td>
<td>[29]</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>Nivolumab</td>
<td>Unselected</td>
<td>0%</td>
<td>0%</td>
<td>14%</td>
<td>N/A</td>
<td>[29]</td>
</tr>
<tr>
<td></td>
<td>BMS-936559</td>
<td>Unselected</td>
<td>0%</td>
<td>0%</td>
<td>5%</td>
<td>N/A</td>
<td>[32]</td>
</tr>
<tr>
<td></td>
<td>Pembrolizumab</td>
<td>Unselected</td>
<td>40% (if MRD)</td>
<td>90%</td>
<td>41%</td>
<td>78% PFS rate @ 20 weeks</td>
<td>[45]</td>
</tr>
<tr>
<td>Anal</td>
<td>Pembrolizumab</td>
<td>PD-L1+ ≥1%</td>
<td>20%</td>
<td>60%</td>
<td>8%</td>
<td>NR</td>
<td>[47]</td>
</tr>
<tr>
<td>Pancreatic</td>
<td>Pembrolizumab</td>
<td>Unselected</td>
<td>0%</td>
<td>0%</td>
<td>5%</td>
<td>N/A</td>
<td>[32]</td>
</tr>
<tr>
<td></td>
<td>Durvalumab</td>
<td>Unselected</td>
<td>8%</td>
<td>25%</td>
<td>13%</td>
<td>NR</td>
<td>[18]</td>
</tr>
<tr>
<td>HCC</td>
<td>Nivolumab</td>
<td>Unselected</td>
<td>23%</td>
<td>69%</td>
<td>17%</td>
<td>72% OS rate @ 6 months</td>
<td>[59]</td>
</tr>
<tr>
<td>Gastric</td>
<td>Pembrolizumab</td>
<td>PD-L1+ ≥1%</td>
<td>22%</td>
<td>36%</td>
<td>10%</td>
<td>24% OS rate at 6 months</td>
<td>[66]</td>
</tr>
<tr>
<td>Breast</td>
<td>Pembrolizumab</td>
<td>PD-L1+ ≥1%</td>
<td>16%</td>
<td>26%</td>
<td>16%</td>
<td>NR</td>
<td>[80]</td>
</tr>
<tr>
<td></td>
<td>Atezolizumab</td>
<td>PD-L1+ &gt;5%</td>
<td>33%</td>
<td>44%</td>
<td>8%</td>
<td>NR</td>
<td>[81]</td>
</tr>
<tr>
<td>NSCLC, squamous</td>
<td>Nivolumab</td>
<td>Unselected</td>
<td>20% regardless of PD-L1 expression</td>
<td>45%</td>
<td>7–14%</td>
<td>↑ OS by 3.2 mos vs docetaxel (HR: 0.59; p &lt; 0.001)</td>
<td>[85]</td>
</tr>
<tr>
<td>Lung</td>
<td>Nivolumab</td>
<td>Unselected</td>
<td>19.2%</td>
<td>44%</td>
<td>10.5%</td>
<td>↑ OS by 2.8 mos vs docetaxel (HR: 0.73; p &lt; 0.001)</td>
<td>[86]</td>
</tr>
<tr>
<td>NSCLC, nonsquamous</td>
<td>Pembrolizumab</td>
<td>Unselected</td>
<td>19.4% (45% if PD-L1+ ≥50%)</td>
<td>46%</td>
<td>9.5%</td>
<td>3.7 months PFS, 12 months OS</td>
<td>[83]</td>
</tr>
<tr>
<td></td>
<td>Atezolizumab</td>
<td>PD-L1+ &gt;5%</td>
<td>45% PD-L1+ (14% PD-L1-)</td>
<td>56% (47%) PD-L1-</td>
<td>11%</td>
<td>89% OS @ 1 year if PD-L1+</td>
<td>[88]</td>
</tr>
<tr>
<td></td>
<td>Atezolizumab</td>
<td>Unselected</td>
<td>38% PD-L1+</td>
<td>NR</td>
<td>43%</td>
<td>OS NR in PD-L1+ (vs 11 months with docetaxel)</td>
<td>[89]</td>
</tr>
<tr>
<td></td>
<td>Durvalumab</td>
<td>Unselected</td>
<td>14% (23% PD-L1+)</td>
<td>42% (48% PD-L1+)</td>
<td>6%</td>
<td>76% ongoing response at 35 weeks</td>
<td>[90]</td>
</tr>
</tbody>
</table>

AE: Adverse event; DCR: Disease control rate; MRD: Mismatch repair deficient; N/A: Not applicable; NR: Not reported; NSCLC: Non-small-cell lung carcinoma; ORR: Overall response rate; OS: Overall survival; PFS: Progression-free survival; RCC: Renal cell carcinoma; SCLC: Small-cell lung carcinoma.
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PD-L1-negative tumors, the combination of PD-1 and CTLA-4 blockade was more effective than either agent alone [103]. Moreover, the presence of pre-existing CD8+ T cells, that are negatively regulated by PD-1/PD-L1-mediated adaptive immune resistance in melanoma, were found to be essential for tumor response to PD-1 blockade [104]. Whether these observations are applicable to other malignancies such as lung cancer remains to be determined.

It has been speculated recently that the number of somatic mutations, leading to a large repertoire of neo-antigens, could explain the clinical responses in some tumors compared with others [105]. This is likely to be one piece in this complex puzzle, given the varying responses to immunotherapy in tumors with similar numbers of somatic mutations. As such, a new form of clinical trials has been created to identify and treat malignancies based on their immune profile, such as the density of TILs and mutational status rather than organ origination (NCT02054806). These ‘basket trials’ may be able to identify predictive biomarkers for response to immunotherapy by analyzing unique T-cell receptor (TCR) phenotypes, immune gene signature and mutational load. Hopefully, this will increase the number of patients who may derive a durable and prolonged response to immunotherapy, such that ‘remission’ may be a more commonly used word.

Future perspective

Without question, immunotherapy will continue to create a paradigm shift in the treatment of various malignancies. The indication of immune checkpoint inhibitors targeting PD-1 and PD-L1 is expected to expand to other disease settings beyond melanoma, lung and renal cancers, as single agents and in combination with other modalities such as chemotherapy and radiation therapy. In addition, it is expected that the next wave of immune checkpoint inhibitors, currently under investigation, will make their way to clinic in the near future. Indeed, cancer immunotherapy will continue to evolve in the next decade with better-defined mechanism of action and novel biomarkers that could be predictive for response.

Financial & competing interests disclosure

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No writing assistance was utilized in the production of this manuscript.

Table 2. Clinical outcomes of PD-1/PD-L1 inhibition in solid tumors other than melanoma (cont.).

<table>
<thead>
<tr>
<th>Cancer type</th>
<th>Drug</th>
<th>Patient population</th>
<th>ORR</th>
<th>DCR</th>
<th>Drug-related AE (grade ≥3)</th>
<th>Survival benefit</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avelumab</td>
<td>Unselected</td>
<td>12% (16% PD-L1+)</td>
<td>50%</td>
<td>12%</td>
<td>PFS 11.7 months PD-L1+ (vs 5.9 months PD-L1-)</td>
<td>[91]</td>
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</tr>
<tr>
<td>Pembrolizumab + Ipilimumab</td>
<td>Unselected</td>
<td>55%</td>
<td>100%</td>
<td>11%</td>
<td>NR</td>
<td>[98]</td>
<td></td>
</tr>
<tr>
<td>Durvalumab + Tremelimumab</td>
<td>Unselected</td>
<td>27%</td>
<td>41%</td>
<td>31%</td>
<td>NR</td>
<td>[99]</td>
<td></td>
</tr>
<tr>
<td>Atezolizumab + platinum doublet</td>
<td>Unselected</td>
<td>67%</td>
<td>87%</td>
<td>7–73% (all grades)</td>
<td>NR</td>
<td>[100]</td>
<td></td>
</tr>
<tr>
<td>SCLC</td>
<td>Nivolumab + Ipilimumab</td>
<td>Unselected</td>
<td>25% (15% nivolumab alone)</td>
<td>55% (37% nivolumab alone)</td>
<td>34%</td>
<td>↑ duration of response (NR vs 6.9 months with nivo alone)</td>
<td>[97]</td>
</tr>
</tbody>
</table>

AE: Adverse event; DCR: Disease control rate; MRD: Mismatch repair deficient; N/A: Not applicable; NR: Not reported; NSCLC: Non-small-cell lung carcinoma; ORR: Overall response rate; OS: Overall survival, PFS: Progression-free survival; RCC: Renal cell carcinoma; SCLC: Small-cell lung carcinoma.
Executive summary

Squamous cell carcinoma of head & neck (SCCHN)
- HPV-associated squamous cell carcinoma of head and neck (SCCHN) have a higher expression of PD-L1 compared with HPV-negative tumors. ORR to anti-PD-1 is 20% regardless of PD-L1 expression or HPV status based on small samples.

Urothelial cancer
- PD-L1 expression correlates to worse overall survival and high-grade pathologic features. In heavily pretreated patients, overall response rate (ORR) was 24% in PD-L1+ tumors treated with PD-1/PD-L1 blockade, with a small number having complete responses.

Renal cell carcinoma
- PD-L1 expression correlates with a more advanced stage, higher grade tumor and worse overall survival. In the second-line setting, anti-PD-1 therapy demonstrated an ORR of 25% with prolonged overall survival compared with everolimus. Combination immunotherapy of anti-PD-1 and anti-CTLA-4 increased ORR but also adverse events (AEs).

Prostate cancer
- Limited data for immune checkpoint expression or inhibition. No response to PD-1 inhibition to date.

Colorectal cancer (CRC) & anal cancer
- PD-L1 and CD8+ T lymphocytes correlate with survival. In patients with metastatic CRC, RR is 2% but increases to 40% if mismatch repair deficient tumors. In anal cancer, ORR of 20% was reported.

Pancreatic cancer
-Suppressive tumor-infiltrating lymphocytes (TIL) and PD-L1 expression correlate to worse prognosis. There are limited data to support the activity of PD-1 and PD-L1 inhibitors in pancreatic cancer.

Cholangiocarcinoma
- Increased TILs correlate with better prognosis. PD-L1 expression correlates with worse survival, advanced pathologic grade and tumor stage. Immune checkpoint inhibitors have not been studied.

Hepatocellular carcinoma (HCC)
- PD-L1 expression correlates to a poor prognosis. In one small study, PD-1 inhibition showed an ORR of 20% with a small number of patients having a complete response.

Gastric cancer
- PD-1 and PD-L1 expression correlates with worse overall survival. PD-1 inhibition has an RR of 20%, with an immune gene signature predicting benefit in a small number of patients.

Breast cancer
- PD-L1 expression is more common in triple negative (TNBC) than ER+ breast cancer. PD-1 inhibition has an RR of 20–30% with rare complete responses in TNBC.

Non-small-cell lung carcinoma (NSCLC)
- PD-L1 expression correlates with worse survival and is predictive of clinical response to PD-1 inhibition in most studies, particularly for adenocarcinoma histology. Response rates range from 15 to 20%, with some studies showing higher PD-L1 expression correlating to a higher response rate of 45%. PD-1 inhibition increases PFS and OS compared with chemotherapy in the second-line setting.

References

Papers of special note have been highlighted as:
• of interest; •• of considerable interest

•• Phase III trial showing anti-CTLA-4 therapy offers durable responses in metastatic melanoma.
•• Phase III trial comparing pembrolizumab to ipilimumab in metastatic melanoma.
•• Phase III trial comparing nivolumab to ipilimumab in metastatic melanoma.
6 Carter L, Fouser LA, Jussif J et al. PD-1/PD-L1 blockade, with a small number having complete responses.
8 Taube JM, Anders RA, Young GD et al. Colocalization of inflammatory response with B7-h1 expression in human melanocytic lesions supports an adaptive resistance.
•• First large trial targeting PD-1 in cancer.
•• Phase III trial comparing nivolumab to everolimus in advanced renal cell carcinoma.
•• First large trial targeting PD-L1 in cancer.


63 Eto S, Yoshikawa K, Nishi M et al. Programmed cell death protein 1 expression is an independent prognostic factor in gastric cancer after curative resection. *Gastric Cancer* 2015 (Epub ahead of print).


Oncotarget

L2 is enriched in high-risk triple negative breast cancer.

amplification of 9p24.1 targeting JAK2, PD-L1, and PD-

expression in triple-negative breast cancer.

Mittendorf EA, Philips AV, Meric-Bernstam F


Gatalica Z, Snyder C, Maney T

and its ligand (PD-L1) in common cancers

Gillanders WE. The presence of programmed death 1 (PD


Review Gentzler, Hall, Kunk et al.

patients progressing after platinum-based chemotherapy. ASCO Meeting Abstracts 33(Suppl. 15), 8034 (2015).


Checkpoints inhibitors for renal cell carcinoma: current landscape and future directions

Immunotherapy with checkpoint inhibitors has arrived and begun to change the landscape of clinical oncology, including for patients with renal cell carcinoma. Specifically, drugs targeting the programmed death 1 and cytotoxic T-lymphocyte associated antigen pathways have demonstrated remarkable responses for patients in clinical trials. In this article, we review the most recent available data for immune checkpoint inhibitors for patients with renal cell carcinoma. We discuss potential strategies for rational combination therapies in these patients, some of which are currently being studied, and address important future considerations for use of these novel agents in the years to come.

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Keywords: cytokine • cytotoxic T-lymphocyte associated protein 4 • immune checkpoint blockade • programmed death 1 • renal cell carcinoma • immunotherapy • VEGF

In 2015 alone, about 61,560 new cases of kidney cancer will be diagnosed in the USA, with an estimated 14,080 kidney-cancer related deaths [1]. These numbers largely reflect cases of renal cell carcinoma (RCC), the most common primary cancer of the kidney. While many tumors are found early and can be cured surgically, approximately 20% present with de novo metastatic disease and about a third of patients diagnosed with localized RCC will ultimately develop metastases [2,3]. The management of metastatic renal cell carcinoma (mRCC) has undergone substantial changes in the past decade, with novel systemic strategies fundamentally altering the approach to this disease.

Prior to 2005, treatment options for mRCC were limited, but evidence existed that RCC might be particularly sensitive to manipulations of the immune system. Everson and Cole were among the first to describe spontaneous remissions in patients with mRCC when they reported cases of tumors exhibiting shrinkage without any substantial treatment in the 1960s [4,5]. IL-2, a cytokine important for regulating circulating lymphocytes, was found to result in durable complete remissions in about 5–7% of clear cell mRCC patients, leading to the approval of aldesleukin by the US FDA for treatment of mRCC in 1992. Unfortunately, most patients did not respond and treatment was extremely toxic, limiting use to experienced, high volume centers and reserved for patients with a good performance status and acceptable comorbid conditions [6,7]. A second cytokine, IFN-α, was also used for this disease, with a modest response rate (RR) of approximately 15% as a single agent, but overall the initial era of immunotherapy for mRCC was characterized by low rates of disease control and high rates of toxicity.

Between 2005 and 2012, seven new drugs garnered approval for the treatment of mRCC. This ushered in the VEGF tyrosine kinase inhibitor (TKI) era, reflecting an enhanced understanding of the biology of mRCC. With the exception of bevacizumab,
these new agents are oral, small molecule TKIs targeting the VEGF. In addition, two drugs targeting the mammalian target of rapamycin (mTOR) pathways to disrupt angiogenesis and intrinsic cell proliferation signals were also approved [8]. By interfering with systems essential for tumor growth and metastasis, combinations of these drugs used in sequence improved the overall survival of mRCC to >28 months [9]. Unfortunately, while initial RR rates are encouraging, most patients ultimately develop resistance and few have durable disease control.

Consequently, the impetus to harness the immune system to combat this disease remained strong. A meta-analysis evaluating tumor response in patients receiving placebo in randomized trials revealed high rates of spontaneous remissions in RCC, validating this approach [10]. More recently, new agents known as immune checkpoint inhibitors that unlock the anti-tumor abilities of the immune system have entered the clinic with promising durable responses in patients across a variety of tumor types, including RCC. The term immune checkpoint refers to the idea that certain pathways inherent to the immune system regulate a sustained immune response and can be stimulated to shut down immune activation. This is in place to protect the host from overzealous immune activity that can result in harm after an insult (be it infectious, malignant or other) is recognized and dealt with. Many tumors have evolved the ability to exploit these pathways to evade immune recognition and maintain undisturbed growth and proliferation. Pharmacologic inhibition of these pathways can restore antitumor control in some patients. Two checkpoint pathways have been successfully targeted in Phase III oncology trials and have led to the approval of new drugs. The programmed death 1 (PD-1) pathway functions peripherally in the tumor microenvironment, while the cytotoxic T-lymphocyte associated protein (CTLA-4) pathway controls early T-cell activation [11]. In this review, we will discuss the agents being used to modify these pathways in the context of RCC, as well as other targets, combinations and strategies being explored to enhance efficacy and advance the treatment paradigm for RCC, commencing a new era of immunotherapy. We will focus on clear cell RCC (ccRCC), the predominant histologic subtype of this disease.

Programmed death 1 pathway blockade
PD-1 is an immunoinhibitory receptor that belongs to the CD28/CTLA-4 family and is inducibly expressed on CD4+ and CD8+ T cells, natural killer (NK) cells, B cells and monocytes within 24 hours from their immunological activation [12]. Usually, activated T-cells, B-cells, NK cells, dendritic cells (DCs) and monocytes express PD-1 in order to restrict autoimmune activity during inflammatory states such as infections. However, tumors have evolved the ability to express the main PD-1 ligand (PD-L1) to exploit this mechanism, thus downregulating the antitumor T-cell response [13]. PD-L1 is not expressed on normal kidney tissues, but is expressed in a significant proportion of both primary and metastatic RCC specimens. [14] Therefore, inhibiting this pathway with monoclonal antibodies (mAbs) targeting either PD-1 or PD-L1 can reenergize exhausted T-cells downregulated by tumor-directed PD-L1 expression, culminating in innate anti-tumor detection and coordinated tumor cell death.

Releasing this restraint on the immune system is not without consequences. The PD-1 checkpoint protects the immune system from overactivation and precipitating autoimmune. Thus, side effects from checkpoint blockade have been characterized by organ-specific inflammatory reactions such as dermatitis and colitis, clinically and pathologically mimicking site-specific autoimmune diseases, and termed immune-related adverse events (irAEs). Serious and unusual toxicities rarely seen with any other agents, such as endocrinopathies and autoimmune pneumonitis, have been described [15]. These toxicities have proven to be a class effect, with severe adverse events (AEs) occurring at a relatively consistent 10–20% rate across clinical trials of PD-1/PD-L1 inhibitors regardless of tumor type. In the landmark Phase III trial evaluating the PD-1 inhibitor nivolumab in patients with previously treated mRCC, grade 3–4 AEs occurred in 19% of patients, but not all of these would be considered irAEs [16]. None of the most common irAEs, such as diarrhea/colitis, occurred as grade 3 or higher in >1% of patients. Nearly all acute autoimmune events have been reversible with prompt initiation of corticosteroids and/or other immunosuppressive agents, and chronic endocrine deficiencies can be managed effectively with replacement therapy [18].

Immune checkpoint inhibitor monotherapy in mRCC
PD-1 blockade
The first immune checkpoint inhibitor to gain FDA approval for patients with mRCC was nivolumab (BMS-936558), a fully human IgG4 monoclonal antibody (mAb) targeting PD-1. Nivolumab has also been FDA approved for the treatment of patients with metastatic melanoma and advanced non-small-cell lung cancer (NSCLC) [17]. Preclinical work by Thompson et al. evaluated PD-L1 expression in ccRCC. In a series of 306 resected tumors, PD-L1 was expressed by 23.9% of the tumors analyzed. Patients whose tumors expressed PD-L1 had a significantly higher rate of metastatic
cancer progression as well as death from RCC [18]. In 2012 results of a Phase I trial with nivolumab were first reported. The trial included patients with various tumor types, including 33 patients with mRCC who had failed conventional agents such as TKIs. There was a 27% objective response rate (ORR) in these patients [19]. Since then several trials studying checkpoint inhibitors specifically in RCC have been reported (Table 1). Notably, the Phase III CheckMate-025 trial demonstrated superior median overall survival (mOS) with nivolumab (n = 406) in patients with advanced RCC who progressed on antiangiogenic therapy compared with patients receiving everolimus (n = 397; 25 vs 19.6 months, respectively; HR: 0.73; p = 0.002) [16]. Nivolumab also showed a trend toward improved progression-free survival (PFS; HR: 0.88; p = 0.11) and fewer patients experienced grade ≥ 3 adverse events (19 vs 37%, respectively). Based on this trial, nivolumab earned approval by the FDA as a second-line therapy following antiangiogenic treatment failure in patients with advanced RCC [20].

Although nivolumab displayed a respectable ORR of 25% in the Checkmate-025 trial, the majority of patients did not respond. There are currently no validated biomarkers for selection of patients who may benefit from nivolumab therapy, but secondary analyses in Checkmate-025 demonstrated that PD-L1 expression on tumor cells was prognostic but not predictive of benefit from checkpoint inhibition. Similarly, an exploratory Phase I study in patients with mRCC receiving three different doses of nivolumab analyzed potential biomarkers of response using four distinct methods: PD-L1 expression, soluble factors from peripheral blood, gene expression profiling and T-cell receptor sequencing [21]. As reported at the 2015 American Society of Clinical Oncology annual meeting, median OS appeared longer in PD-L1 positive patients, but data were not yet mature and PD-L1 negative patients clearly showed responses. While this study suggested PD-L1 expression level may be associated with the probability of prolonged survival, it did not uncover a definitive predictive biomarker. Final efficacy and survival data from this study is expected in early 2016. On the other hand, a study by Sekar et al. showed no correlation between PD-L1 expression and survival in patients with RCC [22].

Pembrolizumab (MK-3475) is a humanized IgG4 PD-1-blocking mAb that has received FDA approval for the treatment of patients with metastatic melanoma and advanced NSCLC after progression on platinum-based chemotherapy [23]. Pembrolizumab was investigated in a Phase I trial enrolling patients with advanced solid tumors, however no patients with mRCC were included [24]. Despite this, and given the robust activity of nivolumab in this disease, pembrolizumab is currently being studied further as a monotherapy in RCC in the neoadjuvant setting (Clinical Trial: NCT02212730) but has not been explored as a single agent in the metastatic setting. As discussed later in this review, pembrolizumab is being studied extensively in combination with other agents in RCC. Table 2 includes ongoing clinical trials of single agents for RCC.

PD-L1 blockade

Given the direct interaction of PD-1 on immune effector cells with PD-L1 on tumor cells, both have proven to be viable targets for pharmacoinhibition. A Phase I trial of the PD-L1 inhibitor BMS-936559 was conducted in patients with varied tumor types, including mRCC, who had failed conventional agents. Seventeen patients with mRCC were enrolled. Objective response was seen in two (12%) and stable disease was seen in seven (41%) patients over 24 weeks [25]. Though this agent is not currently being studied further in mRCC, it demonstrated the potential of PD-L1 inhibition in RCC.

Atezolizumab (atezolizumab, MPDL3280A) is a humanized mAb targeting PD-L1, and results of an expansion cohort of patients with mRCC from its initial Phase I trial have been reported [26]. This trial predominantly enrolled previously treated (57% ≥ 2 prior lines) patients, and also included both clear cell and non-clear cell RCC (nccRCC). Amongst all ccRCC patients assessed for survival (n = 63), the mOS was 28.9 months and the mPFS was 5.6 months. The ORR (n = 62 for this outcome) was 15% amongst ccRCC patients, with no responses by RECIST in the patients with nccRCC (n = 7). Biomarker analysis found no clear association to PD-L1 expression on tumor cells and response. Grade 3 treatment-related adverse events (AEs) occurred in 17% of patients, which was comparable to similar studies of other agents.

At least two other PD-L1 inhibitors have been tested in Phase I studies of patients with advanced solid tumors and have included patients with mRCC, however, efficacy results in mRCC patients have not yet been made available. Recruitment is ongoing for a Phase I trial of the selective human IgG1 anti-PD-L1 mAb durvalumab (MEDI4736, NCT01693562). In this trial, patients with numerous tumor types, including mRCC, are being enrolled. This agent has reported single-agent activity in other tumors, but further studies specific to RCC are noted below with discussion of combination trials. Avelumab (MSB0010718C), a fully human mAb targeting PD-L1, had results of safety and pharmacokinetic data reported at the 2015 American Society of Oncology (ASCO) Annual Meet-
Grade ≥3 AEs were reported in 12.3% of patients, with irAEs reported in 11.7%, both comparable to agents in other trials [28]. Further study of this agent in mRCC is currently limited to combination approaches.

Importantly, PD-1 actually has two main ligands, PD-L1 and PD-L2 [29]. PD-L1 has been more extensively studied and appears to be more diffusely expressed in the immune environment, thus it has been extensively targeted in clinical trials. PD-L1 also binds CD-80 on activated immune cells to impart inhibitory signals, though the clinical relevance of this interaction remains unknown. PD-L2 similarly engages other targets with unclear clinical significance. As drugs inhibiting these targets on both sides of the pathway are tested, these important escape mechanisms need to be considered as sources of differential response kinetics, toxicities and resistance pathways.

**CTLA-4 blockade**

Another prominent immune checkpoint that is expressed on activated T-cells and has been targeted successfully with antineoplastic agents is CTLA-4. This type I transmembrane protein is upregulated on T-cells and functions largely in the lymphatic system as a physiologic safeguard against overactivation of the immune system by outcompeting the T-cell co-stimulatory receptor CD-28 for its ligands CD80 and CD86 to dampen the overall immune response. However, like with PD-1/ PD-L1, many tumor types have developed the ability to exploit this system to circumvent immunosurveillance and avoid a robust antitumor response [30].

Two CTLA-4 directed monoclonal antibodies (mAbs) have been assessed in clinical trials. Ipilimumab was the first immune checkpoint inhibitor made available for clinical use when it received FDA-approval for the treatment of patients with metastatic melanoma in 2011 based on demonstration of a survival benefit in a Phase III trial [31]. It has continued to be studied as a viable single agent in other cancer types, as well as in various combinations which will be covered later in this review. Published data are available from a Phase II trial that investigated two separate dosing schedules of ipilimumab as a single agent in patients with mRCC [32]. This study yielded an ORR of 12.5% in the higher dosed cohort, with six durable PRs reported across the dosing schedules. In a separate analysis of this study focusing on patients who developed enterocolitis as an irAE, the ORR in the mRCC cohort was 35% for patients who developed enterocolitis, compared with 2% in those who did not [33].

Tremelimumab is the other CTLA-4 inhibitor that has been tested in clinical trials, but it has not gained FDA-approval for commercial use, likely due to multiple
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Factors engrained in the design of the definitive Phase III trial in melanoma patients (dosing schedule, criteria used for tumor assessments, patient selection, subsequent treatment options) which failed to meet its primary end point [34,35]. For patients with mRCC, tremelimumab has been evaluated in a Phase I trial in combination with the oral tyrosine kinase inhibitor (TKI) sunitinib [36]. The study enrolled 28 patients, but it was stopped due to excess toxicity (one death and four patients with renal failure) and this combination was aborted. Of 21 patients evaluable for response, 43% achieved a PR.

Although the data in mRCC is limited, it is clear from the melanoma population that CTLA-4 blockade yields a lower ORR and higher toxicity as compared with PD-1 inhibition. Therefore, strategies to increase the number of patients who benefit from checkpoint inhibition are being explored and rational combinations are viewed as the most viable path to success.

### Dual checkpoint blockade

Again extrapolating from studies in the metastatic melanoma population, combined PD-1 and CTLA-4 blockade set the bar for what is possible with immunotherapy. In a three arm, double-blind, Phase III trial, patients with metastatic melanoma were randomized to ipilimumab alone, nivolumab alone, or the combination [37]. The trial met its co-primary end point of improving PFS, which was 11.5 months in the combination arm, compared with 6.9 and 2.9 months, respectively, in the nivolumab and ipilimumab arms (p < 0.001). The ORR in the combination arm was an impressive 57.6%. Unfortunately, with the improved efficacy comes increased toxicity. The incidence of grade 3–4 adverse events was 55.0% in the combination arm, while in the single agent arms grade 3–4 events occurred in 16.3% (nivolumab) and 27.3% (ipilimumab) of patients. More than a third of patients discontinued combination therapy due to toxicity. Notably, responses were noted to endure after cessation of therapy. Accordingly, this combination has gained FDA-approval for patients with metastatic melanoma and is being evaluated in patients with other immunogenic tumors, including RCC.

### Immunotherapy combinations

The capacity for achieving durable responses with checkpoint inhibitors is what drives much of the excitement surrounding these agents. However, the ORR of the nivolumab arm in the Phase III Checkmate 025 trial was a modest 25% [16]. Although ORR is an underestimation of the proportion of patients benefitting from these agents since it cannot account for pseudoprogression, a substantial proportion of mRCC patients do not attain a significant benefit from PD-1 inhibition. Therefore, strategies to increase the number of patients who benefit from checkpoint inhibition are being explored and rational combinations are viewed as the most viable path to success.

### Table 2. Ongoing clinical trials with single agent checkpoint inhibitors in renal cell carcinoma.

<table>
<thead>
<tr>
<th>Trial number</th>
<th>Drug</th>
<th>Setting</th>
<th>Arms</th>
<th>Phase</th>
<th>Expected completion</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT02595918</td>
<td>Nivolumab</td>
<td>Neoadjuvant, nonmetastatic</td>
<td>Single-arm</td>
<td>Pilot</td>
<td>September 2017</td>
</tr>
<tr>
<td>NCT02446860</td>
<td>Nivolumab</td>
<td>Perioperative therapy, first-line metastatic</td>
<td>Single-arm</td>
<td>II</td>
<td>November 2016</td>
</tr>
<tr>
<td>(ADAPTer)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCT02596035</td>
<td>Nivolumab</td>
<td>Second- to fourth-line, metastatic</td>
<td>Single-arm postmarketing</td>
<td>IIIB/IV</td>
<td>October 2017</td>
</tr>
<tr>
<td>(Checkmate 347)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCT02575222</td>
<td>Nivolumab</td>
<td>Neoadjuvant, nonmetastatic</td>
<td>Single-arm</td>
<td>I</td>
<td>December 2017</td>
</tr>
<tr>
<td>NCT02212730</td>
<td>Pembrolizumab</td>
<td>First-line neoadjuvant Prior to cytoreductive nephrectomy</td>
<td>Two arms, randomized to pembro or no presurgical treatment</td>
<td>I</td>
<td>February 2017</td>
</tr>
<tr>
<td>NCT02599779</td>
<td>Pembrolizumab</td>
<td>Second-line or above, metastatic</td>
<td>Pembrolizumab + SBRT given on progression vs SBRT with concurrent Pembro</td>
<td>II</td>
<td>January 2019</td>
</tr>
<tr>
<td>NCT02626130</td>
<td>Tremelimumab</td>
<td>First- or second-line, metastatic</td>
<td>Tremelimumab +/- cryoablation</td>
<td>Pilot</td>
<td>February 2021</td>
</tr>
<tr>
<td>NCT01772004</td>
<td>Avelumab</td>
<td>First-line metastatic</td>
<td>Single-arm dose escalation with RCC expansion cohort</td>
<td>I</td>
<td>April 2017</td>
</tr>
</tbody>
</table>

Checkmate 016 is a Phase I study evaluating various combination regimens in patients with mRCC,
including three dosing combinations of ipilimumab/nivolumab (arm I1: nivolumab 3 mg/kg + ipilimumab 1 mg/kg; arm I3: nivolumab 1 mg/kg + ipilimumab 3 mg/kg; arm IN-3: nivolumab 3 mg/kg + ipilimumab 3 mg/kg). Both treatment naive and previously treated (cytokine therapy only) patients were eligible for enrollment. Updated results from the two arms that progressed to the expansion cohorts (arm IN-3 stopped for excess toxicity) were reported at the 2015 ASCO Annual Meeting [38]. With 47 patients evaluable in each arm, the ORR ranged from 38–43%, with another 40% achieving stable disease (SD) in each arm, thus resulting in a disease control rate (complete and partial responses plus stable disease) approaching 80%. Grade 3–4 toxicities were comparable to those reported in the melanoma study, with 16% of patients discontinuing therapy. The rate of toxicity was highest in the arms with higher ipilimumab dosing. Based on these results, an open-label, randomized, Phase III trial (NCT02231749, Checkmate 214) comparing the ipilimumab/nivolumab combination to sunitinib for patients with previously untreated mRCC has completed accrual.

Combined checkpoint blockade is not restricted to the ipilimumab/nivolumab combination. Several combinations of agents targeting PD-1, PD-L1 and CTLA-4 are being studied in RCC (see Table 3). Pembrolizumab is being evaluated in combination with ipilimumab or the cytokine pegylated IFN-α in a Phase I/II study in patients with mRCC and melanoma (NCT02089685). The dose escalation portion will establish recommended Phase II doses (RP2D) of pembrolizumab with each combination, followed by randomization to each combination or pembrolizumab alone. The PD-L1 inhibitor durvalumab (MEDI4736) is being combined with tremelimumab in a Phase I study of patients with select, advanced solid tumors, including RCC (NCT01975831). Durvalumab is also being studied in combination with MEDI0680 (AMP-514), a PD-1-targeted mAb that also triggers internalization of PD-1 by contacted T-cells, in a Phase I study including all solid tumors (NCT02118337) [39].

Early studies have also begun exploring the inhibition of novel checkpoints in addition to the PD-1 pathway. One example of this is LAG-3, which is an immune checkpoint found on multiple immune cells, including CD8+ T-cells, and binds major histocompatibility complex class II to suppress T-cell proliferation and function [36]. IMP321 is a soluble LAG-3 fusion protein that was tested in a Phase I dose escalation study in patients with mRCC [40]. 21 patients were treated at varying doses, and the drug was well tolerated with grade 1 local reactions being the only clinical side effects and reduced tumor growth noted at higher doses. A Phase I study of the anti-LAG-3 drug BMS-986016 with or without nivolumab in patients with select solid tumors, including RCC, is currently recruiting patients (NCT01968109). Indoleamine 2,3-dioxygenase-1 (IDO1) is not a true immune checkpoint, but is an intracellular enzyme that serves as the rate-limiting step in the kynurenine/tryptophan degradation pathway and has been shown to play an important role in immune tolerance in the tumor microenvironment of many cancers [42,43]. Epacadostat (INCB024360) is an IDO1 inhibitor that is being studied in a Phase I/II study in combination with pembrolizumab in multiple advanced solid tumors (NCT0217822), including mRCC. Preliminary results reported have shown the combination to be well tolerated. Of 19 patients evaluable for response, 15 were reported to have reductions in tumor burden, including an ORR of 40% in the mRCC subset with a disease control rate of 80% [44].

**Combinations of checkpoint inhibitors with VEGF receptor inhibitors**

The standard of care for first-line treatment of mRCC for the better part of the past decade has been with oral TKIs that target the VEGFR, as well as other pathways, to inhibit angiogenesis and suppress tumor growth [9,45,46]. These drugs achieve about a 25–30% response rate in treatment naive patients, with a disease control rate >50%, but rarely result in durable responses, with median PFS ranging from 8.5–11 months [9,45,46]. The TKIs have a manageable and predictable safety profile, with little overlap with the checkpoint inhibitors, thus interest existed to combine approaches. The Checkmate 016 trial, in addition to combining nivolumab with ipilimumab as noted above, also included arms combining nivolumab with a TKI (either sunitinib or pazopanib) in both previously treated and treatment-naive patients. Data for these cohorts was most recently presented at the 2014 ASCO Annual Meeting [47]. In 20 previously treated patients in the nivolumab/pazopanib group, the ORR was 45%. However, 60% of patients experienced a grade 3–4 adverse event, and significant hepatotoxicity led to closure of the nivolumab/pazopanib arm after the first cohort. The nivolumab/sunitinib arms combined (varying by dose of nivolumab) included both previously treated and untreated patients and yielded an ORR of 52%. 73% of patients on this combination experienced a grade 3–4 AE, and though initially this combo showed less hepatotoxicity, effects on liver enzymes were prominent. The significant increase in toxicity of these regimens has discouraged further studies utilizing these combinations. Final results for both toxicity and efficacy are awaited to better understand the value of this combination. Whether altering
Table 3. Ongoing clinical trials with immune checkpoint combination therapies in renal cell carcinoma.

<table>
<thead>
<tr>
<th>Trial number</th>
<th>Drug combination</th>
<th>Setting</th>
<th>Arms</th>
<th>Phase</th>
<th>Expected completion</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT02231749</td>
<td>Nivolumab + Ipilimumab</td>
<td>1st line metastatic</td>
<td>2 arms randomized vs Sunitinib</td>
<td>III</td>
<td>May 2019</td>
</tr>
<tr>
<td>NCT01472081</td>
<td>Nivolumab + (Ipilimumab or Sunitinib</td>
<td>1st or 2nd line metastatic</td>
<td>5 (Nivolumab with each combo and various dose combinations with ipi)</td>
<td>I</td>
<td>February 2016</td>
</tr>
<tr>
<td>NCT02210117</td>
<td>Nivolumab + Bevacizumab or Ipilimumab</td>
<td>Any line metastatic, perioperative cytoreductive nephrectomy treatment</td>
<td>3 (Nivolumab alone and with each combo)</td>
<td>II</td>
<td>November 2018</td>
</tr>
<tr>
<td>NCT02614456</td>
<td>Nivolumab + Interferon-gamma</td>
<td>Metastatic 2nd line and beyond</td>
<td>Single arm dose escalation with expansion RCC cohort</td>
<td>I</td>
<td>December 2017</td>
</tr>
<tr>
<td>NCT01968109</td>
<td>Nivolumab + BMS-986016 (anti-LAG-3)</td>
<td>Metastatic 2nd line and beyond (for RCC)</td>
<td>Single arm dose escalation</td>
<td>I</td>
<td>May 2018</td>
</tr>
<tr>
<td>NCT02089685</td>
<td>Pembrolizumab + Ipilimumab or Pegylated Interferon-alfa</td>
<td>Metastatic 2nd line and beyond</td>
<td>3 (Pembrolizumab alone or with each combo)</td>
<td>I/II</td>
<td>April 2017</td>
</tr>
<tr>
<td>NCT02014636</td>
<td>Pembrolizumab + Pazopanib</td>
<td>Metastatic 1st line</td>
<td>Single arm combo in Phase I; 3 arm randomization to each combo or single agent pazopanib Phase II</td>
<td>I/II</td>
<td>October 2018</td>
</tr>
<tr>
<td>NCT02348008</td>
<td>Pembrolizumab + Bevacizumab</td>
<td>Phase I: Metastatic 2nd line and beyond Phase II: Metastatic 1st Line</td>
<td>Phase I dose escalation of combo, followed by Phase II expansion at MTD</td>
<td>I/II</td>
<td>March 2017</td>
</tr>
<tr>
<td>NCT02133742</td>
<td>Pembrolizumab + Axitinib</td>
<td>Metastatic 1st line</td>
<td>Single arm dose escalation</td>
<td>I</td>
<td>March 2017</td>
</tr>
<tr>
<td>NCT02619253</td>
<td>Pembrolizumab + Vorinostat</td>
<td>Metastatic 2nd line and beyond</td>
<td>Phase I dose escalation of combo, followed by Phase II expansion at MTD</td>
<td>I/II</td>
<td>March 2018</td>
</tr>
<tr>
<td>NCT02178722</td>
<td>Pembrolizumab + INCB024360 (IDO1 inhibitor)</td>
<td>Metastatic 2nd line and beyond</td>
<td>Phase I dose escalation of combo</td>
<td>I/II</td>
<td>May 2017</td>
</tr>
<tr>
<td>NCT01975831</td>
<td>Durvalumab + Tremelimumab (CTLA-4 inhibitor)</td>
<td>Metastatic 2nd line and beyond</td>
<td>Phase I dose escalation of combo</td>
<td>I</td>
<td>October 2017</td>
</tr>
<tr>
<td>NCT02420821</td>
<td>Atezolizumab + Bevacizumab</td>
<td>Metastatic 1st line</td>
<td>Randomized versus sunitinib</td>
<td>III</td>
<td>June 2020</td>
</tr>
<tr>
<td>NCT02174172</td>
<td>Atezolizumab + IFN-alfa (Ipilimumab for NSCLC pts)</td>
<td>Metastatic 2nd line and beyond</td>
<td>Nonrandomized 2 arm trial combining atezolizumab with each combo</td>
<td>I</td>
<td>February 2018</td>
</tr>
<tr>
<td>NCT02493751</td>
<td>Avelumab + Axitinib</td>
<td>Metastatic 1st line</td>
<td>Phase I dose escalation of combo with dose expansion</td>
<td>I</td>
<td>March 2018</td>
</tr>
<tr>
<td>NCT02684006</td>
<td>Avelumab + Axitinib</td>
<td>Metastatic 1st line</td>
<td>Randomized versus sunitinib</td>
<td>III</td>
<td>February 2018</td>
</tr>
<tr>
<td>NCT01441765</td>
<td>Pidilizumab + DC-RCC fusion vaccine</td>
<td>Metastatic any line</td>
<td>Nonrandomized 2 arm trial with pidilizumab alone or the combo</td>
<td>II</td>
<td>Completed accrual</td>
</tr>
</tbody>
</table>
the checkpoint inhibitor or the TKI will make a difference is still a source of interest. Pembrolizumab is being studied in combination with pazopanib in a Phase I/II study in patients with previously untreated patients with mRCC (NCT02014636). The design includes a standard dose, followed by a three arm, randomized Phase II portion looking at the combination versus either drug alone. In a Phase Ib dose finding study, pembrolizumab is also being combined with axitinib, a TKI associated with much less hepatotoxicity, in previously untreated patients (NCT02133742). Preliminary results of the first 11 patients were presented at the 2015 Kidney Cancer Symposium [48]. In regards to toxicity, two patients discontinued treatment due to related AEs and 73% of patients experienced grade 3 AEs, but most were reversible and no patients discontinued treatment due to hepatotoxicity. 6 patients had confirmed PRs and all 11 experienced at least some degree of tumor shrinkage. Enrollment of 55 total patients is now completed with results awaited. Other planned combination trials are listed in Table 3.

Bevacizumab, a mAb that also works to inhibit angiogenesis via inhibition of the circulating ligand VEGF-A, is approved for use in mRCC combined with interferon-alfa based on data from Phase III trials [49,50]. Bevacizumab is not known to be hepatotoxic, so given the initial experience combining the oral TKIs with nivolumab, this combination is attractive. Bevacizumab was investigated in combination with the anti-PD-L1 inhibitor atezolizumab in a Phase I trial with a cohort of patients with mRCC [51]. As presented at the 2015 Genitourinary Cancers Symposium, 12 patients were evaluable for toxicity, 10 for response, with 83% of patients receiving this combination as their first line of systemic therapy in the metastatic setting. No grade 3–4 AEs were attributed to atezolizumab. The ORR was 40%. Currently, atezolizumab is being studied in combination with bevacizumab as an initial treatment for patients with mRCC in Phase II and Phase III studies. In a three-armed, randomized Phase II study, treatment naive patients will receive atezolizumab with or without bevacizumab, or sunitinib as a single agent, until disease progression, with the option to crossover to the combination arm for patients progressing after sunitinib (NCT01984242). This trial has completed accrual with analysis ongoing. A randomized Phase III trial comparing the combination of atezolizumab/bevacizumab to sunitinib is currently open and recruiting (NCT02420821). Combining bevacizumab with pembrolizumab is also currently being tested for safety in patients with mRCC in a Phase I/II trial (NCT02348008).

Checkpoint blockade along with bevacizumab is being explored in a presurgical setting combined with nivolumab. For some patients with mRCC, a cytoreductive nephrectomy is performed based on data from the pre-TKI era demonstrating an overall survival benefit for patients receiving surgery prior to systemic therapy with IFN-α [52,53]. While the utility of this intervention is unproven during the TKI era (prospective trials ongoing), interest may be further re-energized with the emergence of checkpoint inhibition. A three-arm, randomized, Phase II study is currently accruing patients to evaluate the role of nivolumab with or without bevacizumab or ipilimumab prior to planned cytoreductive nephrectomy in patients surgically eligible (NCT02221017).

**Combinations of checkpoint inhibitors with cytokines & vaccines**

Given the dynamic nature of the immune system, there is interest in exploring exogenous ways of priming or manipulating the immune milieu to favor a synergistic response when combined with checkpoint inhibition. One strategy being explored in multiple studies harks back to a previous iteration of treatments for mRCC. IFN-α is a type I cytokine previously in wide use for systemic therapy in patients with mRCC; however, its role has largely been replaced by the TKIs, and now by newer immunotherapies as well. IFN-α has been evaluated in several large trials for patients with mRCC and as a single agent yields an ORR ranging from 7.5–16% [54–56]. This cytokine has also been shown to play an integral role in recruitment and immune recognition during the antitumor response [57]. IFN-γ, the only type II interferon, has a less triumphant role as an anticancer agent, failing to improve survival as a single agent in randomized Phase III trials for various malignancies [58,59]. Nonetheless, its importance in the antitumor immune response is well studied. Dunn and colleagues outlined its many important functions in fostering immunosurveillance in their description of the cancer immunoediting process [60]. Notably, IFN-γ is also known to be a key regulator of PD-L1 expression, and Taube and colleagues have demonstrated that IFN-γ is an essential component of highly PD-L1 enriched tumors [61]. Consequently, combinations of these cytokines with PD-1 pathway inhibitors offer intriguing opportunities to prime the tumor microenvironment and potentially work synergistically to increase efficacy.

As mentioned previously, pembrolizumab is being combined with pegylated-IFN-α or ipilimumab in a randomized Phase I/II study of patients with mRCC or melanoma (NCT02089685). This study is closed to accrual, but not yet reported in RCC. Atezolizumab is being studied combined with conventional IFN-α in a Phase I dose-finding study (NCT02174172).
While this study also includes other tumor types and a combined atezolizumab/ipilimumab arm, the mRCC patients will only be accrued to the arm including IFN-α, and accrual is ongoing. One study is underway utilizing IFN-γ in combination with nivolumab (NCT02614456). This is a Phase I dose-finding study exploring a short course of IFN-γ as induction, followed by combination therapy, in select, advanced tumors with an expansion cohort for patients with mRCC.

Another strategy being employed to prime the immune system includes the use of various cell and vector-based vaccines that attempt to sensitize the immune response to a specific tumor antigen and generate a targeted antitumor response [62]. One current combination involves pidilizumab (CT-011), a mAb against PD-1 derived using an IgGl base to enable antibody-dependent cellular cytotoxicity, along with a dendritic cell-RCC (DC-RCC) fusion vaccine. DCs function as potent antigen presenting cells with host DCs to create a population of DCs that express the entire tumor antigen repertoire, as opposed to one antigen which can more easily be downregulated by the tumor as a defense mechanism [63]. This combination and the PD-1 inhibitor alone are being assessed in a Phase II trial in patients with mRCC during any line of therapy (NCT01441765). The study has completed accrual, but has not yet reported results.

**Adjuvant & neoadjuvant settings**

Most of the trials discussed thus far have been evaluating patients with mRCC. While the durability of responses seen in some patients in this setting is encouraging, there is little expectation that most, if any, of these patients will be truly ‘cured’ of their cancer. On the other hand, the potential to apply the theories of checkpoint inhibition to the setting of early stage disease, with the goal of initiating and maintaining a lifelong antitumor response after resection of a localized mass, is intriguing as a strategy to increase the cure fraction among patients with localized high-risk RCC.

Currently, no FDA-approved therapy exists for the adjuvant treatment of patients with early stage RCC. Despite their success in the metastatic setting, the efficacy of oral TKIs has not translated to improved outcomes when administered after primary surgery, most notably highlighted by the disappointing results of the ASSURE trial evaluating adjuvant sorafenib and sunitinib [64]. With the advent of checkpoint inhibitors, the logical next step is to determine whether these agents could have a role in this setting. However, there are important biological implications to consider. First, the mechanism of action with checkpoint inhibition relies on the presence of tumor antigens to be recognized and targeted by immune cells. After a resection of a primary tumor mass, there may be little, if any, tumor antigen remaining, which may subject patients to the toxicity of therapy without a clear mechanism for benefit. Furthermore, there is evidence that after surgical resection of a kidney tumor, PD-1 expression on immune cells is rapidly and significantly reduced, potentially eliminating the target of a PD-1 inhibitor [65]. These issues have created uncertainty regarding the optimal design of purely adjuvant studies using checkpoint inhibitors, and thus far none have been opened for accrual.

A rational strategy to circumvent some of these concerns is neoadjuvant administration instead of, or in addition to, adjuvant use. This design allows for increased tumor antigen burden at the initiation of treatment to maximize desired immune recognition. It enables a more objective measure of response radiologically, while also enriching pathologic assessments pre and post treatment to support correlative biomarker exploration. Accordingly, multiple trials are currently planned using this design. Nivolumab is being evaluated in two separate, small, single arm trials in slightly different patient populations (NCT02575222, NCT02595918). Pembrolizumab is also being studied in one trial in the neoadjuvant setting, and this trial has begun accruing patients (NCT02212730). Data from these trials will be mostly explorative and hypothesis-generating to support further randomized designs as questions clearly remain. Some of these include the optimal number of doses and time planned preoperatively, whether postoperative treatment should be offered, and whether it could be administered in a brief or extended maintenance fashion. However, since (neo)adjuvant trials take a long time to produce results, the combined Eastern Cooperative Oncology Group and American College of Radiology Imaging Network (ECOG-ACRIN, EA) is in the final stages of planning a large, multicenter, two-armed, randomized, Phase III perioperative trial, The EA8143 (PROSPER trial) has been granted National Cancer Institute approval and will compare two doses of nivolumab followed by surgery and post-op nivolumab for nine months, versus surgery followed by observation alone, in high-risk RCC patients.

**Future perspective & questions**

While the FDA-approval of nivolumab marks the beginning of what may prove to be the new immunotherapy era in the treatment of mRCC, it does inject some uncertainty into the evolving treatment dogma.
The approval of nivolumab in mRCC is in the second-line metastatic setting, after progression on a first-line TKI. This establishes a new standard of care for most patients with mRCC to receive either sunitinib or pazopanib as their initial treatment, followed by nivolumab in the second line, as it has demonstrated a clear OS benefit compared with everolimus in this setting [16]. Based on the path of prior oncologic agents, one might expect the next logical step would be a comparison of nivolumab against a TKI in the first line setting. However, that study has not been planned. Instead, the dual checkpoint blockade combination of ipilimumab/nivolumab is currently the immunotherapy regimen being evaluated in comparison to sunitinib for treatment-naive patients. If this study meets its primary endpoint, where does that leave us? Does ipilimumab/nivolumab become the de facto treatment of choice for all patients in the first line setting? Importantly, we know from the preliminary results from the Checkmate 016 trial, as well as from the melanoma population, that ipilimumab/nivolumab significantly increases the toxicity compared with PD-1 inhibition alone. How much excess toxicity are we willing to accept in the name of improved ORR, especially when some patients may achieve similar benefit, at a fraction of the risk, with single agent nivolumab?

This is where biomarkers may truly become indispensable. Early hope was high that PD-L1 expression on tumors would identify tumors that would respond to PD-1 inhibition, but subsequent studies have clearly demonstrated that PD-L1 expression, whether on the tumor or stromal cells, is not by itself sufficient to predict benefit from PD-1 inhibition in RCC. RCC trials to date have consistently illustrated that while high PD-L1 expression can enhance the likelihood of response, PD-L1 deficient tumors can still respond to treatment. In fact, the Checkmate 025 trial was the first immunotherapy trial to find no difference in efficacy between the PD-L1 positive and negative populations. While much of the focus of biomarker discovery in modern immunotherapy trials has focused on correlating with response, the true power may come from being able to predict which patients can derive benefit from PD-1 pathway inhibition alone, and which patients need combination therapy. Additionally, as it is likely that several combination strategies will demonstrate some clinical benefit, meticulous and rational biomarker assessments embedded in ongoing trials is crucial to be able to categorize patients by the strategy most likely to benefit them. It is our opinion that both single agent and dual checkpoint blockade strategies will ultimately develop a niche in this disease, with biomarkers utilized to help identify patients that can ‘get away with’ single agent therapy versus those that require dual therapy and the associated increased toxicity risks. The progress of some of the alternative combination strategies discussed may further segregate the current homogeneity of the mRCC patients towards more personalized approaches based on biomarkers and tumor and/or host immune biology.

As more checkpoint inhibitors yield benefits in patients, both alone and in combination, having more options for patients is undoubtedly an advantage. But questions regarding sequencing, optimal dosing, duration and treatment schedules, maintenance versus intermittent treatment strategies, and patient selection remain. The potential emergence of checkpoint inhibition in early stage disease may have a trickle-down effect as well. If neoadjuvant or adjuvant therapy demonstrates clinical benefit, what options will we have for those that do relapse? Will they still respond to immunotherapy upon retreatment in a recurrent metastatic setting, or will they need a “boost” from another agent? What if a patient develops a significant irAE in the adjuvant setting necessitating treatment discontinuation? Are they forever excluded from potentially life-altering therapy upon relapse, or can they be rechallenged? These are difficult questions without clear answers at this early stage, but ones that need to be considered and addressed. Supporting innovative clinical trial designs may be needed to help answer some of these, and many other, important questions. These could include sequential therapy trials, designs comparing adjuvant immunotherapy versus immunotherapy upon relapse, continuous versus intermittent PD-1 blockade with redosing at relapse, and rechallenging responders who stop drugs for toxicity and later relapse. Schematic examples of some of these designs are represented in Figure 1.

Summary & conclusion
Checkpoint inhibition has made its mark in the treatment of RCC and the future is bright. Nivolumab is now FDA-approved and available to patients as a standard of care, with more drugs undoubtedly on the way. PD-1 inhibitors offer the chance for durable remissions in a meaningful subset of patients and with a generally manageable toxicity profile. However, the majority of patients still do not achieve a clinical response and more work needs to be done for this resistant/refractory group. Rational combination approaches with TKIs, novel checkpoint inhibitors, and other immune modulators, offer some hope for improved efficacy. Additionally, extending the benefits we have begun to see in the metastatic setting to early stage disease presents a new opportunity for possible cures. We must continue to support clinical trials in mRCC, particularly those with strong preclinical foundations,
and we must safely incorporate high yield correlatives when possible to maximize our knowledge for future trial designs and biomarker candidates. It is an exciting time to study and treat RCC, but the excitement may have really just begun.

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No writing assistance was utilized in the production of this manuscript.

References
Papers of special note have been highlighted as:
• of interest; •• of considerable interest


• This article represents a comprehensive overview of the role of immune checkpoints in cancer and how pharmacologically targeting these checkpoints can fundamentally alter the management of cancer.


• Landmark paper that established the role of the PD-1 inhibitor nivolumab in the treatment paradigm for metastatic renal cell carcinoma (RCC).


• Landmark paper that provided the first evidence that immune checkpoint blockade, in this case with CTLA-4 inhibition, could improve survival for the management of cancer in the setting of metastatic melanoma.


34 Ribas A, Kefford R, Marshall MA et al. Phase III


**Landmark paper that first reported the promising efficacy of dual check-point blockade in a melanoma population.**


Landmark paper establishing the concept of the three phases of immunosurveillance in the control of cancer in the human host.


64 Haas NB, Manola J, Uzzo RG et al. Initial results from ASSURE (E2805): adjuvant sorafenib or sunitinib for unfavorable renal carcinoma, an ECOG-ACRIN-led, NCTN Phase III trial. ASCO Meeting Abstracts 33(Suppl. 7), 403 (2015).


Colorectal cancer (CRC) remains the third most common cause of cancer death in the USA. Despite an increase in the repertoire of treatment options available for CRC, median overall survival has plateaued at approximately 2.5 years. Strategies that engage the patient’s native immune system to overcome checkpoint inhibition have proven to be promising in subsets of CRCs, specifically those with mismatch repair deficiency. Further studies are required to determine combinations of standard therapies with immunotherapy drugs and to discover the best biomarkers to predict response. This review provides insight into the progress made in treating patients with advanced CRC with immunotherapeutics and the areas that demand further research to make these drugs more effective in this patient population.

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Keywords: checkpoint inhibition • colorectal cancer • immunotherapy

Background
Colorectal cancer (CRC) is the third most commonly diagnosed cancer in the USA with an estimated 50,000 deaths in 2015 [1]. Approximately 20% of patients present with advanced metastatic disease that is treatable but not curable. Advances in both cytotoxic chemotherapeutics and targeted agents have resulted in improved outcomes with median survivals reaching almost to 3 years. Regardless, less than 10% of patients experience long term survival and resistance to all known therapies eventually develops. Therefore, new strategies to prolong survival in these patients are still warranted and lessons can be learned from the success of alternative approaches in other tumor types.

The body’s immune system consists of a balanced milieu of stimulatory and inhibitory cells that works to both prevent tumorigenesis and destroy active cancer cells (Figure 1). In CRC, the presence of tumor-infiltrating lymphocytes (TILs) has for many years been associated with improved overall survival (OS) [2]. TIL-containing tumors are more likely to demonstrate microsatellite instability, with recent data revealing strikingly high response rates to immune checkpoint inhibitors [3].

The pivotal role of the immune system in both the surveillance and destruction of tumors has been exploited to produce new treatment options that have garnered much success in melanoma and non-small-cell lung cancer. Specifically, monoclonal antibodies that target and block immune checkpoint proteins allow for the activation of the patient’s innate immune system to fight tumors internally. Despite promising preclinical data that support harnessing the immune system to fight CRC, gaps of knowledge remain in terms of which patients will demonstrate clinical benefit when treated with immunotherapies, and how to incite an immune response in the majority of CRCs where current immune-based therapies seem to be inactive. This article will review the current landscape of immune checkpoint inhibitors for CRC.
inhibition in treating advanced CRC with a focus on further research that is required for increased efficacy of these strategies in CRC.

**Immune surveillance in CRC**

The concept of immune surveillance of cancer cells was originally hypothesized by Paul Erhlich in 1909 [4]. Unfortunately, subsequent studies failed to support this hypothesis. For example, athymic nude mice (entirely lacking T-cells) bred in the 1970s failed to generate increased levels of carcinogen-induced or spontaneously arising tumors relative to mice with intact immune systems [5,6]. Later studies, however, elucidated that the nude mice used in these experiments still had a small population of functional T-cells as well as a normal population of NK cells that likely explained the previous results [7,8], keeping the idea of immune surveillance alive. Finally, in 2001 the immune surveillance concept was demonstrated in a mouse model by Shankaran et al. using Rag-2-null mice, which lack the recombination activating gene and are thus unable to undergo V(D)J rearrangement. He obtained mice completely deficient of T and B lymphocytes and with more unstable and less functional NK cells [9,10]. In these mice, he noted significant increases in methylcholanthrene-induced tumors that were rejected when transplanted into immunocompetent wild-type mice. Rag-2-null animals also showed a significant increase in spontaneous malignant carcinomas. Interestingly, the majority of spontaneous tumors (4 of 6) were colonic in origin [9]. This effect was even more evident when Rag-2-null x γ null mice were examined. These mice lacked all lymphocytes and consequently had no adaptive immune system and a diminished innate immune system. Increased methylcholanthrene-induced tumor burden was seen in these

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**Figure 1.** The pivotal balance of the immune milieu in both stimulating and inhibiting the immune system to both prevent cancer formation and destroy tumor cells.
animals when compared with Rag-2-nulls and there was a higher frequency of rejection when cells from the Rag-2-null x γ c null mice were injected into wild-type mice [8]. Finally, several studies have observed increased rates of cancers (many of which were nonviral or mediated) among severely immunocompromised humans [11,12]. Similarly, registries of solid-organ transplant patients have also shown a small but statistically significant increased risk of CRC [12-14].

Understanding immune surveillance in CRC requires appreciation of the interaction between the tumor and the immune system in a process referred to as immunoediting [15]. Current theories propose that under constant selective pressure from the immune system some tumors develop heterogeneous resistance mechanisms to escape immune destruction [16]. The mechanisms by which this process occurs relies on a complex interplay between the cells of the immune system and tumor cells. The so-called ‘Three E’s’ model of immunoediting theorized that this process occurs in three phases: the elimination phase during which the immune system destroys cancer cells; the equilibrium phase during which growth of the tumor cells and immune-mediated tumor cell death exist in balance manifested by a latency period; and finally the escape phase where tumor progression and hence clinical evidence of cancer occurs [17]. Increasing evidence supports an important role of both the innate immune system as well as the adaptive immune system in CRC immune surveillance. Given the complex interplay between immunomodulators it is helpful to examine each cell type individually.

Macrophages

Macrophages are present at all stages of tumor progression. They develop from circulating monocytes and are recruited to tumors by chemokines such as CCL2, as well as the growth factors CSF-1 and VEGF [18]. They are referred to as tumor associated macrophages (TAMs) and are present within the tumor itself and classically exist in two distinct phenotypes: the so-called M1 and M2 phenotypes (although transcriptome analysis suggests there may be more overlap between the two classes than previously thought) [19,20]. The M1 cells secrete proinflammatory cytokines (IL-6, IL-12, IL-23 and TNF-α), are directly tumoricidal through nitric oxide generation and promote adaptive immunity by increasing MHC1 expression and T-cell costimulatory molecules (CD86 and CD40) [21,22]. M2 cells work primarily to scavenge cellular debris, induce angiogenesis, and hence have an increased tendency to promote tumorogenesis. In human CRC samples, TAMs are often found around necrotic areas of tumor and the advancing margin. Their prognostic significance in CRC has been controversial but seems to portend a positive prognosis [21,23]. Zhang et al. conducted a meta-analysis of 55 studies representing several cancer types and found that only in the five CRC studies included (representing 1149 patient samples) did a high density of TAMs correlate with increasing overall survival [24]. Also Zhou et al. examined a series of 160 cases of stage IIIB and stage IV CRC and found that infiltration of macrophages at the invasive front of the tumor edge was associated with an improved 5-year overall survival and lower levels of hepatic metastases [25]. While the ratio of M1/M2 populations does not seem to differ based on survival or stage, a study of about 450 CRC samples did observe a significant correlation between M1 and M2 markers and improved prognosis. Additionally, a statistically significant inverse correlation with stage was seen for both M1 and M2 expression [26].

Natural killer cells

Natural killer (NK) cells also play an important role in innate immunosurveillance. NK cells have two classes of receptors that work in concert to screen cells for potential malignant transformation: the NKG2D receptor that binds to ligands expressed on tumor cells, and the human killer cell immunoglobulin-like receptor (KIR) that recognizes MHC-1 molecules on cells. The receptor binds to cells with stress-induced ligands presented in their MHC-1. These ligands are thought to function as ‘immuno-alarmers’ and are often present in cells that have undergone malignant transformation. Studies have shown that there are a wide variety of ligands that bind to this receptor with significant redundancy, which is thought to help counter tumor immunoevasion, but the affinities of the ligands to the receptor vary widely [27,28]. KIR receptors exist on the surface of NK cells and recognize MHC-1 molecules. Cancer cells often downregulate expression of surface MHC-1 molecules and NK mediated killing of cells with low levels of MHC-1 is thought to be another form of immune surveillance [29]. In humans, increased levels of intratumoral NK cells have been associated with increased overall survival [30].

Tumor-associated neutrophils

Recently, the role of neutrophils in the immune system’s surveillance of CRC has been investigated. Similar to TAMs these so-called tumor-associated neutrophils (TANs) seem to exist in two subsets: the tumor inhibiting N1 subtype and the tumor promoting N2 subtype with elevated levels of peritumoral TGF-β levels appearing to select for the N2 phenotype [31]. These cells are recruited to sites of tumorigenesis through IL-8, and has been shown to be induced by mutant
KRAS [32,33]. In clinical studies of CRC, higher levels of TANs are correlated with more advanced clinical stage and decreased overall survival [34,35]. Additionally, several studies have shown that an elevated peripheral blood neutrophil to lymphocyte ratio (NLR) is associated with poor prognosis [36]. Specifically, Chua et al. showed that an NLR > 5 was an independent predictor of poor overall survival in a study of 171 patients with mCRC receiving first-line palliative chemotherapy.

Myeloid-derived suppressor cells
The myeloid-derived suppressor cells (MDSCs) are a group of immature macrophages, granulocytes and dendritic cells present in lymphoid and tumor cells [37]. They have been shown to inhibit NK and T-cells through production of reactive oxygen species and nitric oxide (NO), induction of regulatory T-cells (Treg) and enhanced TGF-β secretion [38]. In vitro models of MDSCs indicate direct cell-to-cell contact as an important step in this process [38,39]. In CRC tumor samples an increased proportion of MDSCs in the tumor was correlated with increased metastasis and more advanced tumor stage [37,38].

T-cells
Likely the most critical step in inducing the adaptive immune response to tumor is T-cell activation, a process that is tightly regulated and better understood in recent years. Activation of cytotoxic CD8+ T-cells (CTLs) is dependent on at least three signals: antigenic stimulation through the T-cell receptor (TCR); co-stimulation through molecules such as CD28, CD40, 4–1BB, CD27, ICOS and/or OX40; and stimulation through receptors for inflammatory cytokines, especially IL-12 and IFNα [40]. Once activated, CTLs clonally expand and mediate cellular destruction through direct lysis or apoptosis using perforin and granzyme B [41]. CTLs are critical in detecting the so-called neoantigens that arise as a consequence of tumor-specific mutations as well as tumor-associated antigens (TAAs), classified as normal self-antigens expressed in abnormal concentrations in tumor cells [42,43]. Intracytoplasmic staining combined with cytokine release assays have demonstrated that CTLs found in tumors (TILs) do indeed bind to TAAs in human CRC [44]. Several studies have shown a positive correlation between increased quantity of TILs in the tumor and improved prognosis [45–47]. Mlecnik et al. examined 599 CRC specimens and found that high levels of memory CD8 T-cells (CD8+/CD45RO+) in the tumor epicenter and invasive margin were more accurate at predicting disease-free survival than traditional TNM classifications [46]. Efforts are ongoing to translate these data clinically into a companion to standard staging practices, named the Immunoscore, which can be used to better prognosticate cancers that have been resected [48]. In another study of 959 CRC samples, increased TILs were associated with lower levels of vascular, lymphatic or perineural invasion. Further analysis of these samples showed the TILs from tumors without evidence of local invasion were enriched for mature CD8 cells compared with TILs from tumors where local invasion had occurred [49]. The improved prognosis in patients with tumors that demonstrate increased TILs (which often include tumors with mismatch repair deficiency) is thought to result from suppression of micrometastases through the adaptive immune response, accounting for their improved prognosis.

CD4+ T-cells bind to antigens presented in the MHC-II molecule and further differentiate into helper T-cells (Th1, Th2 or Th17) or Tregs. The CD4+ T-cell differentiation pathway is directed by cytokines. Specifically IL-12 induces Th1 phenotype, IL-4 induces Th2 phenotype, TGF-β and IL-6 induce Th17 phenotype, and TGF-β induces Tregs [50]. Th1 cells secrete IL-2, IFNγ and TNF-α and have been shown to promote CTL proliferation and antibody-dependent cell-mediated cytotoxicity (ADCC) in CRC [49]. IL-4 induces the Th2 phenotype, which promotes tumor growth through secretion of IL-4, IL-5, IL-10 and IL-13. Notably, secretion of Th1 cell cytokines suppresses Th2 cell production and vice versa and this so-called ‘Th1/Th2 shift’ is considered a major cause of escape from immune response [45,51,52]. In CRC, higher expression of genes associated with a Th1 phenotype correlated with improved survival whereas the Th17 cell signature (proinflammatory cells that produce IL-17) portended a poorer prognosis [53].

Tregs are induced by TGF-β inhibited by IL-6, and express CD4, CD25 and Foxp3 [54]. Functionally they serve to diminish the activation of other T-cell populations through direct cell contact and secretion of adenosine, IL10 and TGF-β resulting in net immunosuppression [54,55]. In mouse models, removal of Tregs results in rejection of transplanted tumors [56]. In patients with CRC, increased numbers of Tregs have been demonstrated both in peripheral blood, tumor-draining lymph nodes and tumor tissue [57,58]. Higher stages of CRC and larger tumor size have also been shown to correlate with increased intratumoral expression of Foxp3 [59].

Modes of immune escape in CRC
As mentioned previously the constant selection pressure placed on transformed cells may result in expansion of clones resistant to immunoeediting. The mechanisms by which CRC cells accomplish this is an area of active
that CD73 interacts with both stromal and inflammatory cells to create a metastatic niche that is immune-privileged and predisposes to metastasis [74].

**Clinical data on checkpoint inhibitors**

**CTLA-4**

Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) is a cell surface receptor found on T-cells that, when bound to its ligands (CD80 and CD86) on antigen-presenting cells, works to downregulate the immune system [75]. In addition, crosslinking of CTLA-4 also results in apoptosis of activated T cells, which further impairs their innate function [76]. While CTLA-4 exhibits homology with the co-stimulatory receptor CD28 and binds the same ligands (albeit at much higher affinity), the expression of CTLA-4 raises the activation threshold of T cells, reducing the potency of CD28 activity and hence paving the way for tumorigenesis to take place [77]. Therefore, targeting CTLA-4 as a means of enhancing T-cell-mediated tumor responses represents an attractive treatment strategy.

Polymorphisms within the CTLA-4 gene have been identified, which may dampen the inhibitory effects of the receptor and increase cancer susceptibility. A meta-analysis of 1180 cases of CRC paired with 2110 controls found a significant association between the +49A/G polymorphism and risk of developing CRC with an odds ratio of 1.69 [78]. This amino acid substitution is theorized to result in decreased mRNA levels and thus less CTLA-4 protein production.

The inhibitory effects of CTLA-4 on immune system function are best illustrated in CTLA-deficient mice, in which a rapid lymphoproliferative process occurs within weeks resulting in severe myocarditis and pancreatitis and early death [79]. Subsequent studies performed by Allison et al. showed that blocking CTLA-4 activity with monoclonal antibodies led to decreased tumor growth in mouse models of CRC [80]. Additionally, mice initially treated with anti-CTLA-4 antibodies exhibited either delayed tumor growth or no new tumors at all upon re-challenge of tumor cells, compared with untreated controls that developed significant tumor burden at a rapid rate. Splenocytes isolated from mice treated with anti-CTLA-4 antibodies show an increase in cytotoxic tumor-specific T-cells with an increase in interferon (IFN)γ secretion suggestive of a Th1 response [81]. Synergy has also been illustrated with anti-CTLA-4 antibodies and chemotherapy, specifically ixabepilone and paclitaxel, in a subcutaneous cell line xenograft model of CRC, with a similar induced memory response [82].

Tremelimumab is a fully human monoclonal antibody (mAb) targeting CTLA-4 that in Phase I studies resulted in complete responses in melanoma...
patients and has since been studied alone and in combination with chemotherapy in multiple tumor types [83]. Given strong preclinical rationale supporting a role for CTLA-4 blockade in CRC, a single-arm Phase II trial of tremelimumab in refractory metastatic CRC was conducted in 47 patients [84]. Of the 45 evaluable patients, 95% had disease progression with only one patient exhibiting a partial response. While this result was not considered promising, studies are ongoing with both tremelimumab and ipilimumab (another antibody antagonist of the interaction between CTLA-4 and its ligands) in combination with PD-1 inhibitors in this disease (NCT02408861 and NCT01975831).

**PD-1/PD-L1**

The programmed cell death protein 1 (PD-1) is a receptor found on the surface of T-cells, B-cells and macrophages. PD-1 binds two ligands, PD-L1 and PD-L2. The role of PD-1 in negatively regulating the immune system has been solidified in PD-1-deficient mice, in which a lupus-like proliferative syndrome spontaneously develops with evidence of arthritis and glomerulonephritis [85]. CD8+ T-cells harvested from the spleens of mice lacking PD-1 demonstrate significantly increased activation and proliferation. While the ligands for PD-1 are constitutively expressed on T and B cells, as well as antigen presenting cells, multiple cancers have been reported to upregulate PD-L1. This results in decreased susceptibility of tumor cells to T-cell-mediated attack and hence increased tumor development [86].

Approximately 35% of mismatch-repair (MMR) proficient and 30% of MMR deficient CRC samples have been reported to demonstrate strong expression of PD-L1 via immunohistochemical staining [87]. In MMR proficient CRC, the presence of PD-L1 was associated with lower T-stage, lower histologic grade, lack of lymph node involvement and less vascular invasion. Additionally, improved OS in univariate analysis (HR 0.84, p = 0.003) was demonstrated with a 5-year survival rate of 35% in tumors lacking PD-L1 expression compared with 62% in strong PD-L1 expressing tumors. A direct correlation was also noted between tumors that expressed PD-L1 and the presence of CD8+ T-cells within the tumor infiltrate, suggesting that activation of the innate immune system results in improved outcomes.

Preclinical data exist that suggest a role for targeting the PD-1 pathway in CRC. In a mouse model of CRC in which Colon-26 cells were subcutaneously injected into the flank, intraperitoneal treatment with anti-PD-1 mAb resulted in decreased tumor growth compared with control [88]. Additionally, an increase in the expression of activating cytokines, including IFN-γ and tumor necrosis factor (TNF)α, was observed in mice treated with anti-PD-1 mAb compared with controls. Interestingly, a synergistic effect was seen with dual inhibition of the vascular endothelial growth factor receptor 2 (VEGFR2) compared with either VEGFR2 or PD-1 inhibition alone with a decrease in tumor vascularization but no conflicting effect on T-cell infiltration. A similar additive effect was seen in mice harboring CRC tumors treated with MEDI4736, an anti-PD-L1 mAb, in combination with oxaliplatin, a chemotherapeutic used widely in the treatment of metastatic CRC [89]. This led to an increase in high mobility group box 1 (HMGB1), a marker of immune-mediated cell death and in increase in intratumoral T-cell infiltration.

Phase I trials of both anti-PD-1 and anti-PD-L1 mAbs have been published, and lend credence to the benefit of immune checkpoint inhibition in multiple tumor types [90,91]. A total of 37 patients with CRC were included in these studies with objective responses in patients with non-small-cell lung cancer (NSCLC), melanoma and renal cell carcinoma but none in CRC patients. A larger Phase I study of the anti-PD-L1 mAb MPDL3280A, which mainly enrolled NSCLC, RCC and melanoma patients, did include six patients with refractory CRC with one partial response noted in a patient whose tumors demonstrated increased PD-L1 expression [92]. The combination of MPDL3280A and the anti-VEGF mAb bevacizumab was also evaluated both as a doublet (Arm A) and in combination with 5-FU and oxaliplatin (FOLFOX, Arm B) in a trial of 44 patients with mCRC [93]. Patients in Arm A with refractory disease demonstrated an overall response rate (RR) of 8% whereas treatment-naive patients in Arm B had responses of 44%. The combination was well tolerated with no unexpected toxicities.

While inhibitors of the PD-1/PD-L1 axis have been largely unsuccessful in CRC, data are emerging that patients whose tumors possess deficient MMR proteins with microsatellite instability (MSI) have increased response rates to immune checkpoint inhibitors. This was first noted in a Phase I study of pembrolizumab in 23 CRC patients presented at European Society of Medical Oncology (ESMO) 2015 in which one patient exhibited a partial response and was known to harbor a tumor with MSI [94]. Indeed, a retrospective review of primary CRC tumors found that tumors with MSI, compared with MSS tumors, demonstrated higher expression of five checkpoint proteins (PD-1, PD-L1, LAG-3, IDO and CTLA-4) [95]. Based on the observation that MMR-deficient CRCs harbor TILs far more frequently than
MMR-proficient tumors, the KEYNOTE-164 study evaluated pembrolizumab in three groups of patients: one with MSI-H mCRC, one with MSS mCRC and a third with MSI-H noncolorectal cancers [3]. Validating the investigators’ hypothesis, patients with MMR deficient CRC showed a partial RR to treatment of 40% compared with 0% in patients with MMR proficient CRC with a hazard ratio for disease progression or death of 0.10 (p < 0.001) that favored those with MSI. These tumors possessed a significantly increased number of mean somatic mutations (1782 vs 73) with the authors suggesting that the enhanced presence of neoantigens results in improved responsiveness to PD-1 inhibition. Trials are ongoing investigating the effects of combining immune checkpoint inhibitors with both cytotoxic agents and targeted small molecules in CRC, specifically a study of FOLFOX plus pembrolizumab and a trial combining MPDL3280A with the MEK inhibitor cobimetinib, both in refractory CRC (Table 1).

CD40
CD40 is a transmembrane receptor that is expressed on the surface of antigen presenting cells and is involved in the stimulation of the immune system [96]. The CD40 ligand (CD154) is a member of the TNF receptor family and is found primarily on activated T-cells. Upon binding, increased expression of the MHC results in the leakage of cytokines that both prime activated T-cells as well as recruit activated NK cells. Small studies of human CRC samples have shown CD40 expression rates of about 35% with the infiltration of CD40+ macrophages in CRC demonstrating an advantageous prognosis [97,98]. In mouse models of CRC, treatment with a CD40 agonistic antibody resulted in improved survival with NK cells isolated from these mice demonstrating increased cytotoxic activity against tumor cells [99]. Phase I studies have been completed that evaluated the safety and MTD of the CD40 agonist CD-870,893 in combination with carboplatin and paclitaxel, of which one patient with CRC was enrolled [100].

<table>
<thead>
<tr>
<th>Drug name</th>
<th>Trial number</th>
<th>Phase</th>
<th>Therapy</th>
<th>Patient population</th>
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</thead>
<tbody>
<tr>
<td>AVX701</td>
<td>NCT01890213</td>
<td>Phase I</td>
<td>CEA VRP vaccine</td>
<td>Resected stage III CRC</td>
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<tr>
<td>MEDI4736 (anti-PD-L1 mAb)</td>
<td>NCT02227667</td>
<td>Phase II</td>
<td>Single agent MEDI4736</td>
<td>Advanced refractory CRC</td>
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<td></td>
<td>NCT02261220</td>
<td>Phase I</td>
<td>+ tremelimumab (anti-CTLA mAb)</td>
<td>Solid tumors</td>
</tr>
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<td></td>
<td>NCT02586987</td>
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<td>+ selumetinib (MEK inhibitor)</td>
<td>Solid tumors</td>
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<tr>
<td></td>
<td>NCT02318277</td>
<td>Phase I</td>
<td>+ INCB024360 (IDO inhibitor)</td>
<td>Solid tumors</td>
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<tr>
<td></td>
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<td>Phase I</td>
<td>+ MEDI9447 (anti-CD 73 mAb)</td>
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<tr>
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<td>Phase I</td>
<td>+ AZD1775 (wee 1 inhibitor)</td>
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<td></td>
<td>NCT02118337</td>
<td>Phase I</td>
<td>+ MEDI0680 (anti-PD1 mAb)</td>
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<td>MPDL3280A (anti-PD-L1 mAb)</td>
<td>NCT01988896</td>
<td>Phase I expansion</td>
<td>+ combinetinib (MEK inhibitor)</td>
<td>Advanced refractory CRC</td>
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<tr>
<td></td>
<td>NCT02350673</td>
<td>Phase I</td>
<td>+ RO6895882 (IL-2v targeting CEA)</td>
<td>CEA positive solid tumors</td>
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<td>NCT02471846</td>
<td>Phase I</td>
<td>+ GDC-0919 (IDO1 inhibitor)</td>
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<td>RO7009787</td>
<td>Phase I</td>
<td>+ RO7009787 (CD40 agonist)</td>
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<td>+ MOXR0916 (anti-OX40 mAb)</td>
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<td>Phase I</td>
<td>+ RO5509554 (CSF1-R inhibitor)</td>
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<td>Phase II</td>
<td>+ ablation</td>
<td>Advanced CRC</td>
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<tr>
<td></td>
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<td>+ FOLFOX</td>
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<td>Phase I</td>
<td>+ PF-05082566 (4–1BB agonist)</td>
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<td>Phase I</td>
<td>+ INCB24360 (IDO1 inhibitor)</td>
<td>Solid tumors</td>
</tr>
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Table 1. Ongoing clinical trials of checkpoint inhibitors in colorectal cancer.
1013, RO7009789, SEA-CD40 and ChiLob 7/4) are currently in trial development either as single agents or in combination with other checkpoint inhibitors.

**Conclusion**

Our understanding of how the immune system surveilances the body for cancer and how ultimately cancer cells successfully evade the immune system has allowed for the development of a new promising class of drugs that has made great strides in tumors such as melanoma and lung cancer. Early Phase I studies of drugs that target pivotal immune checkpoints, such as CTLA-4 and PD-1/PD-L1, have enrolled only a handful of patients with CRC and results as single agents have been less than robust. Newer data on the role of these agents in MMR deficient CRC, where the mutational burden is higher and hence a more advanced repertoire of neoantigens is present, appear more promising. Trials are ongoing that evaluate whether combining checkpoint inhibitors with cytotoxic chemotherapy or with other targeted agents will prove more effective. Additionally, more data are urgently required to determine the most accurate biomarker to predict response to these agents in CRC.

**Executive summary**

**Colorectal cancer (CRC) remains a common cause of cancer death despite advances in treatment paradigms**

- Long-term life expectancies in patients with advanced disease is rare, begging the question as to what other strategies exist to prolong a plateaued overall survival of approximately 30 months.

**The role of immunosurveillance in the development of CRC**

- The three E’s model of immunoediting theorizes how the immune system shifts from destroying cancer cells, to a latency period in which tumor growth and immune-mediated tumor cell death exist in balance, to an escape phase in which tumor progression occurs.

**Various immune cells contribute to the immunomodulation of CRC**

- Tumor-associated macrophages (TAMs) portend a positive prognosis in CRC with the M1 phenotype secreting proinflammatory cytokines and increasing T-cell co-stimulatory molecules.
- The role of tumor-associated neutrophils (TAN) in CRC is less well defined, but some data suggest a skew toward a protumor N2 phenotype.
- Intratumoral natural killer (NK) cells are associated with increased overall survival.
- Myeloid-derived suppressor cells (MDSCs) induce regulatory T-cells (Tregs) and hence are associated with more advanced tumor stage.
- Cytotoxic T-cells (CTLs) are critical for recognizing neoantigens present due to tumor-specific mutations and binding tumor associated antigens in CRC.

**Modes of immune escape in CRC**

- Reduced HLA-1 expression, which encodes the α chain of the MHC-1 complex, results in decreased disease specific survival.
- The presence of HLA-G, implicated in immune escape in melanoma, leads to apoptosis of activated CD8 T-cells in CRC and portends a poor prognosis.
- In preclinical models, the co-stimulatory molecule B7-H3 inhibits apoptosis of tumor cells and is associated with drug resistance.

**CTLA-4 downregulates the immune system & impairs T-cell activation**

- CTLA-4 polymorphisms increase the risk of developing CRC.
- Tremelimumab is an anti-CTLA-4 mAb that in a Phase II study of refractory CRC patients had less than ideal response. Studies are ongoing with combinations of tremelimumab and other checkpoint inhibitors.

**The PD-1/PD-L1 pathway as a promising target for deficient MMR CRC**

- 35% of mismatch repair (MMR) proficient and 30% of MMR deficient CRC tumors express PD-L1, which is associated with good risk features.
- Phase I studies of both anti-PD-1 and anti-PD-L1 mAbs included 37 CRC patients, none of which demonstrated meaningful response.
- However, in patients with MMR deficient CRC treated with pembrolizumab, a marked increase in response rate of 40% was reported compared with 0% in patients whose tumors were MMR stable.

**Conclusion**

- Further studies are forth coming to better evaluate combinations of chemotherapy with immunotherapy drugs in CRC.
- Sequencing these drugs with targeted agents is also a promising approach.
- The future will likely bring more data on the role of peptide vaccines and adoptive T-cell transfer strategies for CRC.
**Future perspective**

We exist on the cusp of fully understanding the particular subset of patients with CRC that will benefit from immunotherapy and in exactly what sequence these drugs should be given with other agents, including vaccines, targeted drugs, cytokotoxics and even adoptive T-cell transfer. It is now being recognized that targeted drugs, including anti-EGFR and VEGF antibodies, prime dendritic cells for antigen presentation and may represent potential synergistic combinations with immunotherapy [101]. Alternatively, while whole cell vaccines proved to elicit a less than ideal immune response in CRC, vaccines that target specific TAAs appear more promising. An adenovirus based vaccine used to target carcinoembryonic antigen (CEA) in advanced CRC patients demonstrated a median overall survival in the refractory setting of 11 months with no long-term adverse events [102]. Chimeric antigen receptor therapy, in which T-cells are collected from patients and genetically engineered to express specific receptors on their surface, allowing them to recognize tumor specific antigen, is actively being studied in refractory CRC (NCT01218867). Therefore, the promise of immunotherapy existing as a relevant and reliable therapy option for advanced CRC holds true with further understanding of the mechanisms that drive immune escape and the ways in which these drugs can best be manipulated to harness the most effective results.

**Financial & competing interests disclosure**

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**References**

Papers of special note have been highlighted as:  
• of interest; •• of considerable interest


•• Pivotal manuscript that provides insight into a subgroup of patients with colorectal cancer (CRC) whose tumors demonstrate mismatch repair deficiency with increased response rates to PD-1 inhibition.


•• A review article that summarizes the conflicting roles of the immune system in both protecting the host against cancer and allowing for tumor progression via tumor escape.


19 Sica A, Larghi P, Mancino A et al. Macrophage polarization
Review  Paul, O’Neil & McRee


• A review of the different types of immune cells that infiltrate CRC and how those subgroups effect survival.


• This review summarizes the role of antigens that are formed secondary to mutations within tumors (neoantigens) and how the magnitude of the neoantigen load may influence response to immunotherapy.


• An update on using the Immunoscore, a standardized means of evaluating the immune landscape in tumors, and how that can clinically be incorporated into traditional TNM staging.


• A study of almost 1000 CRC samples comparing pathologic parameters with the local immune response.

50 Yoshida N, Kinugasa T, Miyoshi H et al. A High RORγT/CD3 Ratio is a Strong Prognostic Factor for Postoperative


52 Cui G and Florholmen J. Polarization of Cytokine Profile From Th1 into Th2 Along Colorectal Adenoma-Carcinoma Sequence: Implications for the Biotherapeutic Target. Inflamm. Allergy Drug Targets 7(2), 94–97 (2008).


92 Pivotal Phase I study of anti-PD-1 antibody in solid tumors with little to no response in those patients with CRC.


94 Bendell JC, Powderly JD, Lieu CH, Eckhardt SG et al. Safety and efficacy of MPDL3280A (anti-PDL1) in combination with bevacizumab (bev) and/or FOLFOX in patients (pts) with metastatic colorectal cancer (mCRC). J. Clin. Oncol. 33(Suppl. 3), Abstract 704 (2015).


96 This study of pembrolizumab in patients with CRC was interesting in that the one patient who did respond harbored a tumor with microsatellite instability.


98 This manuscript provides scientific rationale for why patients whose tumors possess microsatellite instability may have increased response rates to immune checkpoint inhibitors due to their increased expression of PD-1, PD-L1, CTLA-4 and IDO.


105 This review highlights the effects of many approved targeted agents on the immune system and how combining them with checkpoint inhibitors may result in synergy depending on the sequence.