Abstract. Lung cancer is the leading cause of cancer-related death worldwide. Treatment of non-small cell lung cancer has evolved tremendously over the past decade. Specifically, immune checkpoint inhibitors have become an increasingly interesting target of pharmacological blockade. These immune inhibitors have shown promising results in front-line therapy and after failure of multiple lines, as well as in monotherapy and combination with other therapies. Vaccination in non-small cell lung cancer is also an emerging field of research that holds promising results for the future of immunotherapy in non-small cell lung cancer. This review presents a concise update on the most recent data regarding the role of checkpoint inhibitors as well as vaccination in non-small cell lung cancer.

Lung cancer is the leading cause of cancer-related death in males, and the second leading cause of death in females worldwide (1). Non-small cell lung cancer (NSCLC) accounts for 85% to 90% of all lung cancer. There are different subtypes of NSCLC that are grouped together because, until recently, the approach to treatment as well as prognosis was often similar. These subtypes are squamous cell carcinoma (SqNSCLC), adenocarcinoma, large cell carcinoma and more poorly differentiated variants (2). Squamous cell carcinoma constitutes about 25-30% of all lung cancer. It originates from the bronchial lining, and is often linked to a history of smoking. Adenocarcinoma represents around 40% of lung cancer. It emerges from mucus-secreting cells. While this type of cancer occurs mainly in current and former smokers, it is the most common type of lung cancer occurring in non-smokers. Large-cell carcinoma accounts for about 10% to 15% of lung cancer and tends to grow and spread quickly (1).

Treatment options for NSCLC have evolved tremendously over the past 15 years, especially with the advent of genetic and molecular techniques to characterize the driver mutations at the cellular level. Overall survival (OS) rates from lung cancer have been increasing slowly over the past decade for both men and women. This is mainly due to reduction in smoking over the past 50 years, although the decline in the rates of lung cancer in men started significantly before that in women (3). Several treatment modalities are being used including surgery, radiation therapy, chemotherapy, targeted therapy, laser therapy, photodynamic therapy, radiofrequency ablation, cryosurgery, electrocautery, and watchful waiting. New modalities are being tested in clinical trials and they include immunotherapy, combination therapies and chemoprevention (4). To date, there are no studies that evaluate the best sequence of available therapies, and as such, the choice of therapy is highly personalized and likely depends on the setting in which available drugs were investigated, stage of disease, cytogenetic or molecular profile, performance status, toxicities, and medical comorbidities.

For a long time, lung cancer has been considered to be non-immunogenic. However, after the success of immunotherapies in melanoma (5), there has been great interest and investigation in the immune checkpoint inhibitors in NSCLC. These immune inhibitors have shown promising results in front-line therapy and after failure of multiple lines, as well as in monotherapy and combination...
with other therapies. Vaccination in NSCLC is also an emerging field of research that holds promising results for the future of immunotherapy in NSCLC.

Programmed Cell Death Protein 1 (PD-1) and Its Ligands in NSCLC

PD-1 is a type I transmembrane protein expressed on the surface of macrophages, dendritic cells, and T- and B-cells. Programmed cell death ligand 1 and 2 (PD-L1 and PD-L2, respectively) both act on PD-1 (6, 7). PD-L2 is mainly expressed on macrophages and dendritic cells, limiting its effects on T-cell regulation. However, PD-L1 is expressed on the same cells that express PD-1. Some cancer cells also express PD-L1 (8). Approximately 60% of NSCLCs have PD-L1 expression (9). After the major histocompatibility complex I (MHC I) on the tumor complexes with the T-cell receptor, there is a release of interferon-γ, which up-regulates expression of PD-L1 on the tumor. PD-L1 then binds to the cluster of differentiation 8 (CD8) T-cell PD-1 receptor, resulting in inhibition of the effector T-cell and evasion of the immune system. The currently approved PD-1-targeted agents include nivolumab, pembrolizumab, and atezolizumab. Nivolumab and pembrolizumab are humanized IgG4 monoclonal antibodies (mAbs) against PD-1. Most therapeutic mAbs are IgG1, which results in antibody-dependent T-cell-mediated cytotoxicity. This, however, would cause destruction of T-cells expressing PD-1; therefore, the IgG4 subtype bypasses this adverse effect (8). The anti-PD-L1 mAbs, durvalumab and atezolizumab, are IgG1, but have been designed to prevent antibody-dependent T-cell-mediated cytotoxicity (ADCC).

In addition to their novel mechanisms of action, immunotherapies have been proven to induce unique responses. The current method for assessing progression is by the Response Evaluation Criteria In Solid Tumors (RECIST) (10). It has been noted in studies involving immunotherapies that using this method to assess response is not adequate. While these agents have shown OS benefit, progression-free survival (PFS) benefit may not be seen in comparison to standard chemotherapy. This is thought to be due to tumor infiltration following initiation of immunotherapy that results in an increase in radiographic tumor burden. It has been appreciated that those with radiographic progression, ‘pseudoprogression’, still received benefit of continuing immunotherapy (8, 11). The time to treatment response for the checkpoint inhibitors has been about 2 months (12-16). There are newer assessment tools such as the immune-related response criteria. They have not been routinely utilized as the comparator arm for these immunotherapy studies as in chemotherapy, where progression has been traditionally evaluated with RECIST (17). There is also no standard PD-L1 assay, and at this time, only pembrolizumab has a complementary assay (Dako 22C3 PD-L1) (18).

Monotherapy in Advanced NSCLC

Nivolumab. Nivolumab is a fully human IgG4 mAb that targets PD-1. Nivolumab interferes with the negative signaling triggered by binding of PD-1 to PD-L1 or PD-L2 and thus restores T-cell antitumor function (19,20).

Nivolumab was initially investigated in a phase I study of 122 heavily pre-treated patients with advanced NSCLC who had received one to five systemic regimens in a phase I study, comparing 1, 3 and 10 mg/kg doses every 2 weeks. This study showed that the 3 mg/kg dose was superior, having an objective response rate (ORR) of 32% (21). This dose was established as the optimal dose in another dose-escalation cohort expansion phase I trial that evaluated 129 patients with advanced NSCLC with one to five prior regimens. Median OS was 9.9 months for the entire group, whereas it was 14.9 months in the 37 patients receiving 3 mg/kg nivolumab, 9.5 months in patients with SqNSCLC and 18.2 months in patients with non-SqNSCLC. Only 14% of patients had grade 3-4 treatment-related adverse events (AEs), and the most common was fatigue (3%), and three patients (2%) died of pneumonitis (22).

In a phase II single-arm trial, 117 patients with advanced SqNSCLC who had received two or more prior therapies, nivolumab was administered as an intravenous infusion at 3 mg/kg every 2 weeks until disease progression or unacceptable AEs occurred. 17 out of 117 patients had an objective response, of which 13 (77%) had an ongoing response at the time of analysis and the median duration of response was not reached. Thirty (26%) out of 117 patients had stable disease, with a median duration of 6.0 months and the median OS was 8.2 months. Grade 3-4 treatment-related AEs occurred in 17% of patients; only one patient died of treatment-related pneumonia and one of ischemic stroke (12). Early data from CheckMate 153 also showed a promising ORR and manageable immune-associated toxicities for those patients who had also been treated with at least two prior systemic regimens (13).

As a second-line therapy for stage IIIB or IV SqNSCLC with disease recurrence after one prior platinum-containing regimen, in a phase III study, CheckMate 017, nivolumab was shown to be more efficacious than docetaxel. The median OS was 9.2 months [95% confidence interval (CI)=7.3 to 13.3 months] with nivolumab versus 6.0 months (95% CI=5.1 to 7.3 months) with docetaxel. The risk of death was 41% lower with nivolumab than with docetaxel (hazard ratio=0.59; 95% CI=0.44 to 0.79; p<0.001). At 1 year, the OS rate was 42% (95% CI=34 to 50%) with nivolumab versus 24% (95% CI=17 to 31%) with docetaxel. The response rate was 20% with nivolumab versus 9% with docetaxel (p=0.008). The median PFS was 3.5 months with nivolumab versus 2.8 months with docetaxel (hazard ratio for death or disease progression=0.62; 95% CI=0.47 to 0.81;
advanced stage IIIA/B NSCLC that has not progressed after monotherapy in the front-line setting for advanced NSCLC patients had not yet been reached at the time the abstract was published (24). The median duration of response for the 52 enrolled nivolumab as a consolidation monotherapy for locally advanced or metastatic NSCLC. Nivolumab demonstrated superior OS (HR=0.73; 95% CI= 0.59, 0.89; p=0.00155) and improved ORR (19.2% vs. 12.4%; p=0.0235). The median time to response was 2.1 months for nivolumab. The median PFS favored docetaxel over nivolumab, 4.2 months versus 2.3 months, respectively. However, the rate of progression at 1 year favored nivolumab, with a rate of 19%, which was better than that with docetaxel (8%). However, a subgroup of patients with advanced non-squamous NSCLC with PD-L1 expression less than 1% did not show a benefit of nivolumab to docetaxel (Table I). Despite the lack of superiority in this subgroup with regards to OS, it was not inferior and there were fewer AEs, still making nivolumab a desirable option over docetaxel (15). Following these results, nivolumab was approved by the US Food and Drug Administration (FDA) as second-line therapy for metastatic NSCLC with progression on or after standard therapy. PD-L1 testing was complementary and not required for nivolumab administration.

There are a few promising trials that are investigating the various clinical settings where nivolumab may be efficacious. The NICOLAS trial is now evaluating nivolumab as a consolidation monotherapy for locally advanced stage IIIA/B NSCLC that has not progressed after standard chemotherapy and radiotherapy (23). There is also a phase I trial investigating the role of nivolumab monotherapy in the front-line setting for advanced NSCLC (24). The median duration of response for the 52 enrolled patients had not yet been reached at the time the abstract was published (range=7.64–85.6+ weeks). The median OS was 98.3 weeks (range=1.0–104.4+ weeks).

Pembrolizumab. Unlike nivolumab, which received non-restricted FDA approval, pembrolizumab received accelerated approval with companion diagnostic PD-L1 assay of 1% or more for advanced NSCLC that progressed while on ongoing or after standard therapy. A phase I trial, KEYNOTE-001, enrolled 495 patients receiving pembrolizumab (at a dose of either 2 or 10 mg/kg every 3 weeks or 10 mg/kg every 2 weeks). There were 21% squamous and 75% non-squamous cases in this study. The pooled ORR was 19.4%, with response rates of 18.0% and 24.8% in previously treated and untreated patients, respectively. Median PFS and OS were 3.0 and 9.3 months in treated patients, respectively, compared to 6.0 and 16.2 months in previously untreated patients. The response rates were similar regardless of dose or schedule. Common side-effects that were attributed to pembrolizumab were fatigue, pruritus, and decreased appetite, with no clear difference according to dose or schedule (18). The efficacy was affected by high-level tumor staining of PD-L1, which prompted the phase II/III study KEYNOTE-010 prior to the completion of KEYNOTE-001. Therefore, in KEYNOTE-010, there were still two dosing regimens for pembrolizumab (2 and 10 mg/kg every 3 weeks) compared to docetaxel but there needed to be at least 1% expression of PD-L1. This study looked at PFS and OS for the entire population and those with 50% or more PD-L1 expression. In those with any PD-L1 expression of 1% or more, the two pembrolizumab arms performed similarly in regards to OS (2 mg/kg dosing was 10.4 months and 10 mg/kg was 12.7 months). There was a clear benefit of pembrolizumab over docetaxel, which had a median OS of 8.5 months. The median PFS was about 4 months in all arms of the study. In those with 50% or more PD-L1 expression, the median OS in the 2 mg/kg (14.9 months) and 10 mg/kg (17.3 months) pembrolizumab arms was about double that with docetaxel (8.2 months). The median PFS in this high-expression group was about 5 months for pembrolizumab and 4 months for docetaxel, a statistically significant difference (25) (Table II). Pembrolizumab is also currently being investigated in a phase II trial as a consolidation therapy following initial treatment with concurrent chemoradiation in patients with inoperable or unresectable stage IIIA or IIIB NSCLC (26).

While there is currently a phase II study of pembrolizumab after high-dose radiation versus pembrolizumab alone in patients with advanced NSCLC (27), two other randomized, open-label, phase III studies, KEYNOTE-042 and KEYNOTE-024, have been designed to evaluate pembrolizumab as first-line monotherapy compared to platinum-based chemotherapy in patients with locally advanced or metastatic PD-L1-positive NSCLC (28,29). KEYNOTE-024 results were recently published. The clinical trial compared pembrolizumab (at the fixed dose of 200 mg every 3 weeks with the investigator’s choice of chemotherapy as first-line treatment for patients with advanced NSCLC and 50% or more tumor score for PD-L1. Pembrolizumab was associated with significantly longer PFS and OS. Median PFS was 10.3 months in the pembrolizumab group versus 6 months in the chemotherapy group (p<0.001)

<table>
<thead>
<tr>
<th>PD-L1</th>
<th>Nivolumab</th>
<th>Docetaxel</th>
<th>HR (95% CI)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥1%</td>
<td>17.1</td>
<td>9.0</td>
<td>0.59 (0.43-0.82)</td>
<td>0.06</td>
</tr>
<tr>
<td>&lt;1%</td>
<td>10.4</td>
<td>10.1</td>
<td>0.90 (0.66-1.24)</td>
<td>0.06</td>
</tr>
<tr>
<td>≥5%</td>
<td>18.2</td>
<td>8.1</td>
<td>0.43 (0.30-0.63)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>&lt;5%</td>
<td>9.7</td>
<td>10.1</td>
<td>1.01 (0.77-1.34)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>≥10%</td>
<td>19.4</td>
<td>8.0</td>
<td>0.40 (0.26-0.59)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>&lt;10%</td>
<td>9.9</td>
<td>10.3</td>
<td>1.00 (0.76-1.31)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

PD-L1: Programmed death-ligand 1; HR: hazard ratio; CI: confidence interval.

Table I. Survival analysis in CheckMate 057 (15).
and the estimated rate of OS at 6 months was 80.2% in the pembrolizumab group compared to 72.4% in the chemotherapy group \( (p=0.005) \) (29).

**Atezolizumab.** Atezolizumab is an IgG1 antibody to PD-L1 that has refined to prevent ADCC. It was approved on October 18, 2016 by the FDA for the treatment of patients with PD-L1-positive NSCLC whose cancer had progressed during or after standard treatment. The FDA approval was based on two trials, POPLAR and OAK. In POPLAR, a phase II study, atezolizumab was compared to docetaxel after first-line therapy of NSCLC. Patients were randomly allocated to the atezolizumab or docetaxel groups \( (142 \text{ vs. } 135 \text{ patients}) \). Intention-to-treat analysis showed an OS of 12.6 months with atezolizumab versus 9.7 months with docetaxel \( (p=0.04) \). Increased OS was associated with increased PD-L1 expression \( (30) \). OAK was a phase III study that also compared atezolizumab to docetaxel, finding a median OS of 13.8 months in the atezolizumab group versus 9.6 months in the docetaxel group \( (p=0.0004) \) (31).

A phase II study, FIR, of atezolizumab in PD-L1-selected patients with NSCLC showed clinical efficacy in both chemo-naïve and previously treated NSCLC. High PD-L1 expression was associated with a higher oRR \( (32) \).

A phase II study, BIRCH, is also recruiting participants to observe the response to atezolizumab as first-, second-, and third-line therapy for patients with PD-L1-positive locally advanced or metastatic NSCLC (33).

**Durvalumab.** Durvalumab is another IgG1 antibody directed against PD-L1 that has been designed to prevent ADCC. ATLANTIC, a phase II trial, has been designed to evaluate the efficacy of durvalumab as third-line therapy in patients with locally advanced or metastatic NSCLC (34).

ARCTIC, however, is a global study to assess the effects of durvalumab given as monotherapy or in combination with tremelimumab, determined by PD-L1 expression, versus standard of care in patients with locally advanced or metastatic NSCLC (35).

**BMS-936559.** BMS-936559 is a fully human, PD-L1-specific, IgG4 mAb that inhibits the binding of PD-L1 to both PD-1 and CD80. In a phase I trial for treatment of NSCLC with BMS-936559, there was an ORR of 10%: 5 out of 49 patients with NSCLC, four with non-squamous and one with squamous type. Three of these patients had responses lasting at least 24 weeks. Stabilization of disease at 6 months took place in 12% of patients. In the overall population, grade 3 or 4 AEs occurred in 9% of patients (36).

Overall, and as evident with other immunotherapies, anti-PD-1 medications can be accompanied by a flare response. This is usually the case when the patient is clinically improving but radiographically has evidence of disease progression. This is usually transient and eventually responders will have disease stability or tumor regression. Moreover, in patients who stopped the drug after disease stability or response, response can still be seen if the drug is revisited upon progression (21). Tables III and IV summarize the completed and ongoing clinical trials, respectively, of PD-1/PD-L1 inhibitors as monotherapy in NSCLC.

**Combination with Chemotherapy in Advanced NSCLC**

Combination of PD-1/PD-L1 inhibition with standard chemotherapy has brought new hope to outcomes in advanced NSCLC. KEYNOTE-021 evaluated the safety, tolerability, and clinical activity of pembrolizumab combined with chemotherapy for treatment-naïve advanced NSCLC. Patients with stage IIIB/IV NSCLC and no prior

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**Table II. Survival analysis in KEYNOTE-010 (25).**

<table>
<thead>
<tr>
<th>Pembrolizumab-docetaxel therapy</th>
<th>PD-L1 ≥1%</th>
<th>PD-L1≥50%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pembrolizumab at 2 mg/kg every 3 weeks/docetaxel</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median OS, months</td>
<td>10.4/8.5</td>
<td>14.9/8.2</td>
</tr>
<tr>
<td>HR (95% CI)</td>
<td>0.71 (0.58-0.88), ( p=0.0008 )</td>
<td>0.54 (0.38-0.77), ( p=0.0002 )</td>
</tr>
<tr>
<td>Median PFS, months</td>
<td>3.9/4.0</td>
<td>5.0/4.1</td>
</tr>
<tr>
<td>HR (95% CI)</td>
<td>0.88 (0.74-1.05), ( p=0.07 )</td>
<td>0.59 (0.44-0.78), ( p=0.0001 )</td>
</tr>
<tr>
<td><strong>Pembrolizumab at 10 mg/kg every 3 weeks/docetaxel</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median OS, months</td>
<td>12.7/8.5</td>
<td>17.3/8.2</td>
</tr>
<tr>
<td>HR (95% CI)</td>
<td>0.61 (0.49-0.75), ( p&lt;0.0001 )</td>
<td>0.50 (0.36-0.70), ( p&lt;0.0001 )</td>
</tr>
<tr>
<td>Median PFS, months</td>
<td>4.0/4.0</td>
<td>5.2/4.1</td>
</tr>
<tr>
<td>HR (95% CI)</td>
<td>0.79 (0.66-0.94), ( p=0.004 )</td>
<td>0.59 (0.45-0.78), ( p&lt;0.0001 )</td>
</tr>
</tbody>
</table>

PD-L1: Programmed death-ligand 1; OS: overall survival; HR: hazard ratio; PFS: progression-free survival.
systemic therapy were randomized to pembrolizumab plus carboplatin and paclitaxel (cohort A; any histology) or carboplatin plus pemetrexed [cohort C; nonsquamous without epidermal growth factor receptor (EGFR)-sensitizing mutation or anaplastic lymphoma kinase (ALK) translocation only]. Preliminary ORR was 30% in cohort A and 58% in C (37). Some phase I/II studies are currently designed to investigate pembrolizumab in combination with chemotherapy for patients with advanced NSCLC (38, 39).

Atezolizumab (15 mg/kg intravenously every 3 weeks) in combination with chemotherapy as first-line therapy also showed promising clinical activity with well-tolerated toxicities. ORR was 67% in the 37 patients evaluated. The major drug-related toxicities included grade 3-4 AE of neutropenia (7%), thrombocytopenia (7%), while only one grade 5 AE of candidemia was observed (40). Nivolumab combined with chemotherapy also led to similar outcomes. The results of the CheckMate 012 study of nivolumab plus chemotherapy in advanced NSCLC were as follows: ORR 33-47%, median PFS 21.0-31.0 weeks, and 1-year OS rate 50-87%. No treatment-related deaths occurred, but a treatment-related toxicity rate of 45% at grade 3/4 was reported (16).

### Combination with Targeted Therapy

EGFR tyrosine kinase inhibitors (TKIs), such as gefitinib, erlotinib, and afatinib, are recommended as first-line therapy in EGFR-mutant advanced NSCLC. Combination therapy of PD-L1 pathway blockade and EGFR-TKIs has been shown to be promising by data from preclinical studies. It has been suggested that EGFR activation up-regulates expression of PD-L1, and hence contributes to immune evasion (41, 42). A retrospective study that included 125 patients with NSCLC, including patients with mutant and wild-type EGFR, V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS) and ALK all with PD-L1 expression found a correlation between PD-L1 expression and *EGFR* mutation (43). PD-L1-positive patients had higher sensitivity to EGFR-TKIs than PD-L1-negative patients in terms of the response rate (p=0.01). Remarkably, PD-L1-positive tumors were significantly associated with adenocarcinoma histology (p=0.005). Another retrospective study of 56 patients with EGFR-mutant advanced lung adenocarcinoma demonstrated a significantly higher disease-control rate (p=0.004), longer PFS (p=0.001), and OS (p=0.004) after TKI therapy in PD-L1-positive patients (44). A third retrospective analysis of 170 patients with advanced NSCLC treated with EGFR-TKIs indicated that PD-L1

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**Table III. Clinical trials for programmed cell death protein 1/programmed cell death ligand 1 (PD-1/PD-L1) inhibitors as monotherapy in non-small cell lung cancer (NSCLC).**

<table>
<thead>
<tr>
<th>Study name</th>
<th>Agent</th>
<th>Phase</th>
<th>Stage/line</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>(21)</td>
<td>Nivolumab</td>
<td>I</td>
<td>Advanced or recurrent malignancies including squamous and nonsquamous NSCLC</td>
<td>ORR=18.4%</td>
</tr>
<tr>
<td>CheckMate 063 (12)</td>
<td>Nivolumab</td>
<td>I</td>
<td>Heavily pretreated advanced NSCLC previously treated with ≥2 regimen</td>
<td>ORR=17%, OS=9.9 months</td>
</tr>
<tr>
<td>CheckMate 017 (14)</td>
<td>Nivolumab</td>
<td>III</td>
<td>Advanced or metastatic that progressed during or after ≥1 prior regimen</td>
<td>ORR=20% vs. 9% with docetaxel</td>
</tr>
<tr>
<td>CheckMate 057 (15)</td>
<td>Nivolumab</td>
<td>III</td>
<td>Non-sqNSCLC that progressed during or after Pt-based regimen</td>
<td>ORR=19.2% vs. 12.4% with docetaxel</td>
</tr>
<tr>
<td>KEYNOTE-001 (18)</td>
<td>Pembrolizumab</td>
<td>I</td>
<td>Locally advanced or metastatic</td>
<td>Pooled ORR=19.4%, RR=18.0%</td>
</tr>
<tr>
<td>KEYNOTE-010 (25)</td>
<td>Pembrolizumab</td>
<td>II/III</td>
<td>Previously treated, PD-L1-positive advanced NSCLC</td>
<td>Clear benefit, almost doubling of OS compared with docetaxel</td>
</tr>
<tr>
<td>KEYNOTE-024 (29)</td>
<td>Pembrolizumab</td>
<td>III</td>
<td>Previously untreated, strong PD-L1-expressing stage IV</td>
<td>OS=10.3 months vs. 6 months with chemotherapy, OS at 6 months=80.2% vs. 72.4%</td>
</tr>
<tr>
<td>POPLAR (30)</td>
<td>Atezolizumab</td>
<td>II</td>
<td>Advanced or metastatic after Pt failure</td>
<td>Median OS=12.6 months vs. 9.7 months with docetaxel</td>
</tr>
<tr>
<td>OAK (31)</td>
<td>Atezolizumab</td>
<td>III</td>
<td>Advanced or metastatic after Pt failure</td>
<td>Median OS=13.8 months vs. 9.6 months with docetaxel</td>
</tr>
<tr>
<td>(36)</td>
<td>BMS-936559</td>
<td>I</td>
<td>Locally advanced or metastatic</td>
<td>ORR=10%</td>
</tr>
</tbody>
</table>

ORR: Overall response rate; OS: overall survival; SqNSCLC: squamous; Non-sqNSCLC: non-squamous NSCLC; Pt: platinum; RR: response rate; PFS: progression-free survival.
expression was associated with significantly shorter OS in patients with wild-type EGFR \( (p=0.029) \), but not in those with mutant EGFR \( (p=0.932) \) \( (45) \). Combination therapy of PD-L1 blockade and EGFR-TKIs was investigated in an escalation phase I study where durvalumab was utilized along with gefitinib in two cohorts (cohort A: MEDi4736 at 3 mg/kg every 2 weeks and cohort B at 10 mg/kg every 2 weeks). Data showed that the maximum tolerated dose was not reached and no drug-related toxicities were observed. Grade 3-4 AEs were observed in three patients, including dyspnea/hypoxia, myalgia/fatigue, and elevated alanine aminotransferase level \( (46) \).

Bevacizumab is a recombinant mAb that works as anti-angiogenesis therapy by blocking the vascular endothelial growth factor (VEGF). It is recommended in combination with platinum-doublet chemotherapy for locally advanced or metastatic non-SqNSCLC as first-line therapy or monotherapy maintenance. For patients who have previously responded to bevacizumab, maintenance with bevacizumab as monotherapy is recommended. Data from the AVAPERL study demonstrated that PFS and median OS for patients treated with single-agent bevacizumab were 3.7 and 13.2 months, respectively \( (47) \). A phase I study evaluated the efficacy of nivolumab with and without bevacizumab maintenance in patients with advanced NSCLC which did not progress during or after first-line platinum-based chemotherapy. Median PFS was 37.1 weeks with nivolumab and bevacizumab combination therapy, with the median OS not reached. Few cases of grade 3 treatment-related AEs, but no grade 4 AEs occurred in the combination arms \( (48) \).

Necitumumab is a second-generation, recombinant human IgG1 mAb to EGFR. Two randomized controlled trials, INSPIRE and SQUIRE, indicated that necitumumab with gemcitabine and cisplatin as a first-line therapy improved OS in treatment-naïve patients with advanced SqNSCLC \( (11.5 \text{ vs. } 9.9 \text{ months}; \ p=0.01) \) but not in non-SqNSCLC treated with necitumumab with pemetrexed and cisplatin \( (49, 50) \). Combination therapy of necitumumab and pembrolizumab is currently being studied for safety and efficacy in patients with advanced SqNSCLC and non-SqNSCLC in a phase I study \( (51) \).

**Cytotoxic T-Lymphocyte-associated Protein 4 (CTLA-4) in NSCLC**

CTLA-4 is expressed exclusively on T-cells. Both CTLA-4 and T-cell co-stimulatory CD28 receptor expressed on antigen-presenting cells share the same ligands (CD80 and CD86). However, CTLA-4 has a much higher affinity for both CD80 and CD86, which makes it compete with CD28 in binding to...
these ligands. This results in inhibition of CD28 stimulatory effect on T-cells (52, 53). CTLA-4 regulates the early stage of T-cell activation through additional mechanisms, including recruitment and activation of the Src homology region 2 domain-containing phosphatase-2 (SHP2) and protein phosphatase 2A (PP2A) via the YVKM motif in its cytoplasmic domain. This results in attenuation of kinase signaling, such as phosphatidylinositol-3 kinase/protein kinase B (PKB) also known as Akt pathway, induced by T-cell receptor and CD28. CTLA-4 primarily regulates CD4+ T-cells by down-regulating the activity of T-helper cells and enhancing the immunosuppressive activity of T-regulatory cells (54, 55).

**Ipilimumab.** Ipilimumab is an antibody against CTLA-4 approved for the treatment of metastatic melanoma (56). Its activity is under investigation for NSCLC.

A phase II trial of ipilimumab in combination with chemotherapy published some preliminary results. Patients with previously untreated metastatic NSCLC were randomly assigned to one of three groups: carboplatin-paclitaxel, carboplatin-paclitaxel with concurrent ipilimumab, or carboplatin-paclitaxel with sequential ipilimumab (57). Results were very promising, with a statistically significant improvement in PFS in the sequential arm (carboplatin and paclitaxel for two cycles followed by addition of ipilimumab).

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**Table V. Clinical trials for programmed cell death protein 1/programmed cell death ligand 1 (PD-1/PD-L1) inhibitors as combination therapy in non-small cell lung cancer (NSCLC).**

<table>
<thead>
<tr>
<th>Study name</th>
<th>Agents</th>
<th>Phase</th>
<th>Stage/line</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>AVAPLR (47)</td>
<td>BEV+PEM</td>
<td>III</td>
<td>Advanced non-SqNSCLC</td>
<td>Median PFS improved from 3.7 to 7.4 months</td>
</tr>
<tr>
<td></td>
<td>NVB+BEV</td>
<td></td>
<td>No progression within 42 days of completing ≥4 cycles first-line Pt-based chemotherapy</td>
<td>ORR=8% vs. 10% with NVB alone</td>
</tr>
<tr>
<td>INSPIRE (50)</td>
<td>NEC+PEM-CIS</td>
<td>III</td>
<td>Stage IV NSCLC</td>
<td>No significant difference in OS between treatment groups</td>
</tr>
<tr>
<td>SQUIRE (49)</td>
<td>NEC+GEM-CIS</td>
<td>III</td>
<td>Chemo-naive advanced SqNSCLC</td>
<td>Median OS=11.5 months vs. 9.9 months with GEM-CIS alone</td>
</tr>
</tbody>
</table>

BEV: Bevacizumab. PEM: pemetrexed; NVB: nivolumab; NEC: necitumumab; CIS: cisplatin; GEM: gemcitabine; (non-) SqNSCLC: (non-) squamous NSCLC; PFS: progression-free survival; Pt: platinum; ORR: overall response rate; OS: overall survival.

**Table VI. Ongoing clinical trials for programmed cell death protein 1/programmed cell death ligand 1 inhibitors as combination therapy in non-small cell lung cancer (NSCLC).**

<table>
<thead>
<tr>
<th>Study name</th>
<th>Study identifier</th>
<th>Agents</th>
<th>Phase</th>
<th>Stage/line</th>
<th>Outcomes</th>
<th>Grade 3-4 AEs</th>
</tr>
</thead>
<tbody>
<tr>
<td>KEYNOTE-021 (37)</td>
<td>NCT02039674</td>
<td>Pembrolizumab+Pt</td>
<td>I/II</td>
<td>Unresectable or metastatic</td>
<td>ORR=30% in any histology and 58% in nonSqNSCLC positive for EGFR or ALK</td>
<td>Reversible AST/ALT elevation, anemia, rash, and colitis</td>
</tr>
<tr>
<td></td>
<td>NCT02382406</td>
<td>MK-3475+CPT/Nab-PAC</td>
<td>I/II</td>
<td>Previously untreated advanced</td>
<td>No results yet</td>
<td>No results yet</td>
</tr>
<tr>
<td></td>
<td>NCT02422381</td>
<td>MK-3475+GEM</td>
<td>I/II</td>
<td>Previously treated advanced</td>
<td>No results yet</td>
<td>No results yet</td>
</tr>
<tr>
<td></td>
<td>NCT01633970</td>
<td>MPDL3280A+Pt</td>
<td>I</td>
<td>Locally advanced or metastatic solid tumors Advanced</td>
<td>ORR=67% in first 37 patients ORR=33-47%</td>
<td>Anemia, neutropenia and thrombocytopenia Pneumonitis, fatigue and ARF</td>
</tr>
<tr>
<td>CheckMate 012 (16)</td>
<td>NCT01454102</td>
<td>Nivolumab+Ctx</td>
<td>I</td>
<td>Locally advanced/ metastatic, any EGFR status</td>
<td>Of 7 patients with ≥1 8-week tumor assessment; 3 had reduction in tumor size No results yet</td>
<td>Dyspnea/hypoxia, myalgia/fatigue, and elevated ALT</td>
</tr>
<tr>
<td></td>
<td>NCT02088112</td>
<td>MEDI4736+GEF</td>
<td>I</td>
<td>Locally advanced/ metastatic, any EGFR status</td>
<td>Of 7 patients with ≥1 8-week tumor assessment; 3 had reduction in tumor size No results yet</td>
<td>No results yet</td>
</tr>
<tr>
<td></td>
<td>NCT02451930</td>
<td>Pembrolizumab+ necitumumab</td>
<td>I</td>
<td>Stage IV</td>
<td>No results yet</td>
<td>No results yet</td>
</tr>
</tbody>
</table>

AEs: Adverse events; non-SqNSCLC: non-squamous NSCLC; Pt: platinum-based chemotherapy; Ctx: Chemotherapy; ORR: overall response rate; GEM: Gemcitabine; GEF: gefitinib; EGFR: epidermal growth factor receptor; ALK: anaplastic lymphoma kinase; ALT: alanine aminotransferase; AST: aspartate aminotransferase.
with chemotherapy for four more cycles) compared to the chemotherapy alone arm (median PFS of 5.1 vs. 4.2 months, \( p=0.02 \)). Subset analysis showed a greater benefit in patients with squamous histology and thus, a phase III study of ipilimumab in combination with carboplatin and paclitaxel for squamous cell carcinoma of the lung is ongoing (NCT 02279732) (68). Ipilimumab is also being studied in NSCLC in combination with radiation (NCT02239900, NCT02221739) (59, 60) and with other immunotherapy agents (NCT02039674, NCT02174172) (61, 62).

**PD-1/PD-L1 blockade in combination with CTLA-4 inhibitors.** A phase III trial to evaluate the efficacy and safety of durvalumab as third-line monotherapy (substudy A, PD-L1-positive) plus tremelimumab (substudy B, PD-L1-negative) versus standard of care (including erlotinib, gemcitabine, or vinorelbine) in patients with stage IIIB/IV NSCLC is currently being conducted (34).

**Vaccines in NSCLC**

The role of vaccination in cancer stems from the antigens uniquely expressed on tumor cells. Vaccines have been increasingly studied in NSCLC (63).

The START trial investigated tecemotide (L-BLP25), a vaccine against MUC1 (a transmembrane mucin family protein), in patients with stage III NSCLC who had completed chemoradiation. MUC1 is a tumor-associated antigen overexpressed in lung cancer and other epithelial tumors. MUC1 immunogenicity stems from an aberrant glycosylation of this protein in tumor tissues compared to normal tissues. There were 1,513 patients enrolled without progressive disease randomized to receive either tecemotide or placebo. The primary endpoint of the study was OS. There was no significant difference in survival between the two arms (median OS of 25.6 months with tecemotide versus 22.3 months with placebo), although there was a benefit for the vaccine in patients who received concurrent rather than sequential chemotherapy (30.8 vs. 20.6 months, \( p=0.0175 \)). These promising results were not confirmed in other studies, and development of this compound has been halted (64).

In the MAGRIT trial, patients with surgically resected stage IB-IIIA NSCLC were randomized to receive placebo or a vaccine to melanoma-associated antigen 3 (MAGE-A3). Eligible patients were required to have MAGE-A3-positive tumors. A total of 2,272 patients were randomized and treated. Treatment was well tolerated but results were disappointing as the trial showed no significant improvement in disease-free survival (60.5 versus 57.9 months, \( p=0.7379 \)) (65).

TG4010 is a recombinant modified vaccinia Ankara that codes for MUC1 and interleukin-2 (66). MUC1 encoded by TG4010 shares epitopes with tumor-associated MUC1.

The combination of TG4010 with first-line chemotherapy for advanced NSCLC has been studied in two randomized clinical trials. Findings from these studies were promising (67, 68).

The TIME trial was a phase IIb/III trial to assess TG4010 in combination with first-line chemotherapy. The trial concluded that TG4010 plus chemotherapy seems to improve PFS relative to placebo plus chemotherapy (69).

**Conclusion**

As a result of the success of multiple antibody inhibitors of PD-1/PD-L1 and CTLA-4 in clinical studies, immunotherapy has become an intriguing field for investigation in therapy for NSCLC. These therapeutic modalities have shown efficacy as first-, second-, and even third-line treatment in patients with NSCLC. Further research is needed to determine the best sequence of use of these agents and to identify the unique characteristics of those patients with durable remissions.

**References**


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