Immunotherapies for NSCLC: Are We Cutting the Gordian helix?

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Abstract. Chemotherapy is currently the standard-of-care for non-oncogene-driven advanced non-small cell lung cancer (NSCLC). Due to improvements in chemotherapeutic choices and supportive care, patients currently typically undergo multiple lines of chemotherapy as their disease progresses. Although treatments have improved over recent years, limited benefits are seen, especially in patients receiving later-line chemotherapy, as response rates can be low, response duration short and survival poor. Molecular-targeted therapies have provided improvements in outcomes. However, these treatments only offer a clear benefit in subsets of tumors harbouring the appropriate genomic alteration (mutation, amplification, translocation). Recent advances in immunotherapy have highlighted the potential of immunoncology based treatments for NSCLC, offering the potential to provide durable responses and outcomes regardless of histology or mutation status. Blocking inhibitory pathways such as the cytotoxic lymphocyte antigen-4 (CTLA-4) and programmed cell death-1 (PD-1) checkpoint pathways with monoclonal antibodies has generated antitumor immune responses that are transforming cancer therapeutics. PD-1 and programmed cell death ligand-1(PD-L1) antibodies have shown durable responses in NSCLC, with a favourable safety profile and manageable side effects. The activity of immune checkpoint inhibitors is currently been assessed in treatment-naïve patients with PD-L1–positive advanced NSCLC. Combinatorial approaches with other immune checkpoint inhibitors, chemotherapy, or targeted-agents are being explored in ongoing clinical trials, and may improve outcome in NSCLC. The emerging data not only offer the hope of better cancer therapy but also provide evidence that changes our understanding of how the host immune system interacts with human cancer. It is therefore conceivable that agents blocking the CTLA-4/ PD-1/ PD-L1 axis will provide valuable additions to the growing armamentarium of targeted-agents.

Lung cancer is the leading cause of cancer-related death worldwide (1). Current treatment strategies for non-small cell lung cancer (NSCLC; 80-85% of all lung cancers) include chemotherapy regimens based on histology and targeted-agents for patients who carry specific genomic alterations. Platinum-based chemotherapy, with or without maintenance therapy (based on histology) and subsequently followed by second-line cytotoxic chemotherapy, is the standard treatment for most patients with advanced NSCLC, with a median survival of approximately 1 year (1). The advent of molecularly targeted therapies has, however, dramatically improved outcomes in the metastatic setting for patients with lung adenocarcinomas that harbour somatically activated oncogenes such as EGFR and translocated ALK. However, even with these therapies, the majority of patients with NSCLC do not attain prolonged disease control, and 5-year survival rates remain low. Thus, therapies that obtain long-lasting disease control without significant side effects are urgently needed.

Advances in our understanding over the molecular biology of NSCLC have led to effective and approved targeted agents for tumors with EGFR mutations and ALK or ROS1 rearrangements. Additionally, vascular endothelial growth factor (VEGF), heat-shock protein 90 (HSP90), the mammalian target of rapamycin (mTOR), phosphatidylinositol 3-kinase (PI3K), B-raf, HER2 and RET translocation have also been shown to be additional potential targets of interest (2, 3). New therapies and treatment strategies are required to...
immunotherapy even for tumors that were historically considered as nonimmunogenic (4).

Immuno-oncology is a novel therapeutic strategy currently being evaluated for the treatment of lung cancer. This approach differs from traditional modalities, which target the tumor directly or aim to disrupt the tumor blood supply, as it is designed to potentiate the patient’s immune response to tumor cells. Immuno-therapy is now emerging as a major modality in NSCLC treatment focusing on development of inhibitors of the molecular mediators of cancer-induced immunosuppression (immune checkpoints) to boost antitumor immune responses. Different immunologic approaches targeting immune checkpoint pathways are showing promise in development, and preclinical and clinical evidence provides the rationale for investigating these newer immunotherapies in NSCLC which will be the focus of this review.

**Targets for Immunomodulating Agents**

The immune system is capable of identifying tumor-associated antigens (so-called neo-antigens) and eliminating the cancerous cells expressing them. Expression of these neo-antigens (new epitopes) is regarded to be a consequence of new mutations (e.g., EGFR) and/or DNA damage (4). Immune checkpoints refer to multiple inhibitory pathways that counteract certain crucial steps of T-cell-mediated immunity to maintain self-tolerance and modulate the duration and amplitude of immune responses. Recently, the understanding of several checkpoints that shut down the immune system as an immunosuppressive mechanism in tumors has evoked a paradigm shift in cancer treatment (4, 5).

Immune checkpoints are initiated primarily through T-cell inhibiting and stimulating receptors and their ligands, including cytotoxic T-lymphocyte-associated protein (CTLA), PD-1 (programmed cell death-1) and PD-L1 or PD-L2 (programmed cell death ligand-1,-2; CD273), among many others (CD27, CD40, CD137,OX40, Tim-3, LAG-3 [lymphocyte activation gene-3]) (reviewed in 5, 6). Inhibitory ligands and receptors that regulate T-cell effector functions are commonly overexpressed in tumor cells or in the tumor microenvironment. The blockade of immune checkpoints releases the breaks on the immune system resulting in antigen-specific T-cell responses. The most studied immune checkpoint receptors are the inhibitory receptors CTLA-4 and PD-1.

CTLA-4 (CD152) is expressed on T-cells and regulates the early stages of T-cell activation; it counteracts the activity of the T-cell costimulatory receptor CD28 by competing for its ligands B7.1 (CD80) and B7.2 (CD86). CTLA-4 primarily regulates CD4+ T-cells and enhances the immunosuppressive activity of regulatory T-cells (Tregs). Inhibitors of CTLA-4 (e.g., ipilimumab, tremelimumab) will cause a more than 5,000-fold increase of CD4+ and CD8+ cells within the tumor (7). In contrast, PD-1 (CD279), a receptor of the CD28 family, mediates immune resistance in the tumor microenvironment by downregulating the activity of effector T-cells in peripheral tissues in the setting of an inflammatory response (8). PD-1 is expressed on tumor-infiltrating lymphocytes (TIL; mainly CD4+ T-cells) as well as on B-cells, natural killer cells, monocytes, and dendritic cells. Upon binding to its ligands (PD-L1 and PD-L2), PD-1 inhibits kinases that are involved in T-cell activation; PD-1 is highly expressed on Treg cells and may enhance their proliferation. PD-1 engagement on the T-cell surface leads to phosphorylation of PD-1 intracytoplasmic tyrosines. It also increases SH2-domain-containing tyrosine phosphatase 2 (SHP-2) associations with the immunoreceptor tyrosine-based switch motif (ITSM) of PD-1. Recruitment of SHP-2 de-phosphorylates signaling through the PI3K pathway and downstream signals through Akt. Therefore, PD-1 stimulation upon T-cell receptor ligation ultimately decreases the induction of cytokines, such as IFN-γ, and cell survival proteins, such as Bcl-xL, and decreases T-cell proliferation and survival. In addition, IFN-γ has been shown to stimulate PD-L1 expression on tumor cells suggesting that response to anti-PD-1 antibodies might be more pronounced in patients with high IFN-γ levels (9). Recent studies have revealed that PD-L1 is widely expressed in various types of cancers, providing a foundation for the development of PD1/PD-L1 antibodies as cancer immunotherapy (8, 9).

The major function of PD-1 is to limit the activity of T-cells in peripheral tissue during an inflammatory response and to limit autoimmunity. Thus, the interaction of PD-1 with its ligand PD-L1 plays an important role in the regulation of T-cell activation, peripheral tolerance, and immunopathology (8).

PD-L1 is a key molecule of the immune checkpoint in cancer cells, that, by binding to PD-1 on T-cells, attenuates antitumor immunity, facilitating the escape of tumor cells from the T-cell-mediated immune response. PD-L1 (CD274) is part of a complex system of receptors and ligands that are involved in controlling T-cell activation (10) (Figure 1).

Targeting PD-L1 could, therefore, have additional effects within the tumor microenvironment via binding to PD-1, and acts later in the process of T-cell activation. PD-L1 expression also reflects the role of the tumor immune
microenvironment in response to anti-PD-1 therapy (12-14) (Figure 2). In melanoma, infiltrating CD8+ T-cells have been shown to upregulate PD-L1 expression using a mechanism independent of the oncogene-driven process (15, 16). However, targeted-therapies can modulate the T-cell immune response in the tumor microenvironment. Thus, activation of the immune checkpoint in tumors most likely stems from an interplay between the oncogene (tumor cells) and T-cells (microenvironment) (17). PD-L1 has also been demonstrated to correlate with the poor prognosis of patients with several types of cancer, including esophageal cancer, renal cell carcinoma, hepatocellular carcinoma, pancreatic cancer, colorectal cancer, gastric cancer, and NSCLC. In contrast, PD-L1 expression was shown to correlate with better prognosis in breast cancer (18, 19).

Understanding how the immune system affects cancer development and progression has been one of the most challenging questions in immunology. It is now generally accepted that the immune system plays a dual role in cancer: it cannot only suppress tumor growth by destroying cancer cells or inhibiting their out-growth but also promote tumor progression either by selecting for tumor cells that are more fit to survive in an immunocompetent patient or by establishing conditions within the tumor microenvironment that facilitate tumor out-growth. This concept is called “cancer immunoediting” (initially described by Schreiber et al.) and integrates the immune system’s dual patient-protective and tumor-promoting roles (20). According to Schreiber et al. cancer immunoediting is an extrinsic tumor suppressor mechanism that engages only after cellular transformation has occurred and intrinsic tumor suppressor mechanisms have failed. In its most complex form, cancer immunoediting consists of three sequential phases: elimination, equilibrium, and escape (20). In the elimination phase, innate and adaptive immunity work together to destroy developing tumors long before they become clinically apparent. Many of the immune molecules and cells that participate in the elimination phase have been identified, but more work is needed to determine their exact sequence of action. If this phase goes to completion, then the patient remains in complete remission, and elimination thus represents the full extent of the process. If, however, a rare cancer cell variant is not destroyed in the elimination phase, it may then enter the equilibrium phase, in which its outgrowth is prevented by immunologic mechanisms. T-cells, IL-12, and IFN-γ are required to maintain tumor cells in a state of functional dormancy, whereas NK cells and molecules that participate in the recognition of effector function of cells of innate immunity are not required (20); this indicates that equilibrium is a function of adaptive immunity only. Editing of tumor immunogenicity occurs in the equilibrium phase. Equilibrium may also represent an end stage of the cancer immunoediting process and may restrain out-growth of occult cancers for the lifetime of the patient. However, as a consequence of constant immune selection pressure placed on genetically unstable tumor cells held in equilibrium, tumor cell variants may emerge that (i) are no longer recognised by adaptive immunity (antigen loss variants or tumors cells that develop defects in antigen processing or presentation), (ii) become insensitive to immune effector mechanisms, or (iii) induce an immunosuppressive state within the tumor microenvironment (20). These tumor cells may then enter the escape phase, in which their out-growth is no longer blocked by immunity. Several mechanisms have been implicated in this phase: T-cells that are exhausted express high levels of PD-L1 (anti-PD-1/PD-L1 agents may therefore restore exhausted T-cells). In addition, recent data indicate that exhaustion is maintained/controlled by DNA methylases. Moreover, preclinical studies suggest that caspase-8 (most importantly), β-2-microglobulin, and MHC I and II are mutated, and the PD-L1 genes are amplificated (confers resistance to anti-CTLA-4/PD-1/PD-L1 antibodies) (21). These tumor cells emerge to cause clinically recurrent disease (Figure 3).

Table 1. Summary of clinical data correlating PD-L1 expression with clinical response rates (Percentages state PD-L1 cutoff threshold).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Nivolumab</th>
<th>Atezolizumab</th>
<th>Durvalumab</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Assay used</strong></td>
<td>28-8</td>
<td>SP142</td>
<td>SP263</td>
</tr>
<tr>
<td><strong>Cutoff point</strong></td>
<td>1-5%</td>
<td>1-10%</td>
<td>25%</td>
</tr>
<tr>
<td>ORR in PD-L1</td>
<td>31% (5%; 1st line)</td>
<td>31% (1%; 1st line)</td>
<td>26% (25%; 2nd line)</td>
</tr>
<tr>
<td>positive tumors</td>
<td>13% (1%; 2nd line)</td>
<td>46% (5%; 2nd line)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15% (5%; 2nd line)</td>
<td>83% (10%; 2nd line)</td>
<td></td>
</tr>
<tr>
<td>ORR in PD-L1</td>
<td>10% (1st line)</td>
<td>20% (1st line)</td>
<td>10% (25%; 2nd line)</td>
</tr>
<tr>
<td>negative tumors</td>
<td>14-17% (2nd line)</td>
<td>18% (2nd line)</td>
<td></td>
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Inhibition of the CTLA-4 and the PD-1/PD-L1 pathways has shown to enhance intratumoral immune response in numerous preclinical studies (22, 23), and blockade of immune checkpoints has introduced a new era in cancer treatment. Response rates with these inhibitors in NSCLC are evolving (Table I); and it becomes clear that a substantial proportion of patients achieve clinically meaningful and prolonged PFS.

Response patterns with immunotherapy differ from those of cytotoxic agents, and ORR and PFS traditionally used as clinically-meaningful end-points seem to be limited in their ability to predict outcome. Immune-related response criteria (irRC) have been introduced to characterize patterns of response and novel clinical endpoints such as the irPFS, defined as the time of initiation of treatment to immune-related progression or death, are used, however, are not universally accepted (24). Given the late plateau in survival curves with immunotherapy driven by long-term responders, OS remains the golden standard primary endpoint.

Patient selection for immunotherapy and identification of predictive biomarkers for immune therapies are currently active areas of research. PD-L1 expression by immunohistochemistry has been found to predict response to PD-1 and PD-L1 inhibitors, conferring increased ORR to pembrolizumab (25), atezolizumab (MPDL3280A) (26, 27), and durvalumab (MEDI4736) (28) (Table I). However, in

Figure 1. Tumor immunology and the PD-1/PD-L1 pathway (modified after 11).
free survival (irPFS) with ipilimumab, when ipilimumab was given after chemotherapy (5.7 months versus 4.6 months, \( p=0.05 \)) (29). The rationale for administration of chemotherapy before ipilimumab (phased regimen) was to allow antigen release to occur before initiation of immune modulation with ipilimumab. There was a trend toward improved OS in patients who received phased chemotherapy with ipilimumab compared with chemotherapy alone (12.2 months versus 8.3 months, \( p=0.23 \)). PFS and OS benefit were more prominent for squamous cell carcinomas (HR, 0.55; 95% CI, 0.27-1.12 and HR, 0.4; 95% CI, 0.2-1.03, respectively). Common toxicities included anemia, diarrhea, and fatigue; grade 3/4 immune-mediated toxicities (colitis, transaminitis, and hypophysitis) occurred more commonly in patients receiving ipilimumab (29). A phase III confirmatory trial is ongoing (NCT01285609).

Tremelimumab (AstraZeneca, Manchester, UK) is a fully human IgG2 anti-CTLA-4 immunoglobulin monoclonal antibody. A phase II study of tremelimumab as maintenance therapy compared with observation in patients with stable or responding disease after first-line chemotherapy failed to show an improvement in PFS (30). Tremelimumab is also currently under investigation in a phase IIb trial for the treatment of patients with PD-L1-negative tumors, albeit at a lower rate, can still respond to these antibodies. The use of PD-L1 expression as a companion diagnostics platform for immune therapies has been limited by the lack of a standardised assay, different cutoff points to determine PD-L1 positivity, variability in intervals between biopsy and treatment, as well as sample preservation.

**Monotherapy Data**

Ipilimumab (Yervoy®, Bristol-Myers Squibb, Princeton, NJ, USA) is a fully humanised IgG1 anti-CTLA-4 monoclonal antibody that blocks binding of CTLA-4 to its ligand. A randomised phase II clinical trial of paclitaxel and carboplatin with or without ipilimumab in treatment-naïve stage IV NSCLC showed improvement in immune-related progression-free survival (irPFS) with ipilimumab, when ipilimumab was given after chemotherapy (5.7 months versus 4.6 months, \( p=0.05 \)) (29).
second-line and third-line treatment of unresectable pleural or peritoneal mesothelioma (NCT01843374).

The safety and clinical efficacy of nivolumab (Opdivo®, Bristol-Myers Squibb, Princeton, NJ, USA), a fully human IgG4 monoclonal antibody directed against the PD-1 receptor, were evaluated in a phase I study in previously treated (up to 5 prior lines of therapy; >50% received >3 lines) patients with a range of solid tumors, including patients with advanced squamous and non-squamous NSCLC. ORR was 17% and median duration of response was 17.1 months in patients with NSCLC, with similar ORRs recorded in PD-L1 positive and PD-L1 negative patients (15% and 14%, respectively). Follow-up indicated median OS of 9.9 months, with median 1-, 2- and 3-year OS rates of 42%, 24% and 18%, respectively, for all patients. Median OS was 14.9 months for patients receiving 3 mg/kg nivolumab, with 1-, 2- and 3-year OS rates of 56%, 42% and 27%, respectively. Nivolumab had an acceptable safety profile; adverse events (AEs) were generally manageable using systemic glucocorticoids and/or other immunosuppressive agents. Similar median OS rates were reported for squamous and non-squamous subtypes (9.2 and 10.1 months, respectively) and PD-L1 positive and PD-L1 negative patients (7.8 and 10.5 months, respectively). Clinical activity (ORR) was also observed across all patient subgroups, including those who had received <3 (12%) and ≥3 (21%) prior therapies and those with or without EGFR (17% and 20%, respectively) or K-ras (14% and 25%, respectively) mutations. Sub-group analysis suggested that a history of smoking was associated with significantly improved response; ORR was 0% in patients with a smoking exposure <5 pack-years versus 30% in those with an exposure >5 pack-years (31). A single-arm phase II study of nivolumab (CheckMate 063; NCT01721759) in patients with advanced or metastatic squamous NSCLC who have received at least two prior systemic regimens reported an ORR of 17%, with 76% of responses ongoing at the time of analysis. Median OS was 8.2 months and 1-year OS was 42% after a median follow-up of 8 months. Clinical activity was observed in PD-L1-positive and PD-L1-negative patients (32). A subsequent randomised, open-label, international, phase III study evaluated the efficacy and safety of nivolumab, as compared to docetaxel in advanced squamous-cell NSCLC who had disease progression during or after first-line chemotherapy (CheckMate 017, NCT01642004) (33). The median OS was 9.2 months (95% CI, 7.3 to 13.3) with nivolumab versus 6.0 months (95% CI, 5.1-7.3) with docetaxel. The risk of death was 41% lower with nivolumab than with docetaxel (HR, 0.59; 95% CI, 0.44-0.79; p<0.001). At 1 year, the OS rate was 42% (95% CI, 34-50) with nivolumab versus 24% (95% CI, 17-31) 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 (HR for death or disease progression, 0.62; 95% CI, 0.47-0.81; p<0.001). The expression of the PD-L1 was neither prognostic nor predictive of benefit (33). Nivolumab was subsequently approved for the treatment of metastatic squamous NSCLC with progression on or after platinum-based chemotherapy. A randomised phase III study of nivolumab (3 mg/kg every 2 weeks) versus docetaxel (75 mg/m² every 3 weeks) (n=582) in patients with non-squamous NSCLC (CheckMate 057; NCT01673867) was stopped early after an independent monitoring panel determined the primary endpoint of improved OS had been reached. Eligible patients will now be allowed to continue treatment or cross over to the nivolumab arm in an open-label extension of CheckMate-057 (34).

In a most recently published study patients (n=129) with heavily pretreated advanced NSCLC received nivolumab 1, 3, or 10 mg/kg intravenously once every 2 weeks in 8-week cycles for up to 96 weeks. Median OS across doses was 9.9 months; 1-, 2-, and 3-year OS rates were 42%, 24%, and 18%, respectively, across doses and 56%, 42%, and 27%, respectively, at the 3 mg/kg dose (n=37) chosen for further clinical development. Among 22 patients (17%) with objective responses, estimated median response duration was 17.0 months. From this study it was concluded that nivolumab monotherapy produced durable responses and encouraging survival rates even in patients with heavily pretreated NSCLC (35).

Pembrolizumab (Keytruda®, Merck&Co., Kenilworth, NJ, USA) is an anti-PD-1 humanised IgG4 monoclonal antibody. In a phase I study of 38 previously treated patients with advanced NSCLC receiving pembrolizumab, ORR was 21% using RECIST and 24% using irRC. The median PFS reported for pembrolizumab-treated patients was 9.1 weeks, with a median OS of 51 weeks (25). In a most recently published phase I study of first-line pembrolizumab in 45 patients with locally advanced or metastatic NSCLC (KEYNOTE-001, NCT01295827), ORR was 36% by irRC and 52% of patients experienced a drug-related AE. Pooled analysis of treatment-naive and pretreated patients (n=262) receiving pembrolizumab showed an ORR of 21% using RECIST and 23% using irRC; ORR was 23%/25% in patients with PD-L1-positive tumors and 9%/13% in patients with PD-L1-negative tumors. Grade 3-5 drug-related AEs were observed in 9% of patients (36). Pembrolizumab is currently being tested versus docetaxel (NCT01905657) and platinum-based combinations for PD-L1–positive NSCLC (NCT02142738) in previously-treated and metastatic treatment-naive NSCLC, respectively. A phase III trial comparing pembrolizumab with platinum-doublet chemotherapy in treatment-naive PD-L1–positive patients is ongoing (NCT02220894). The activity of pembrolizumab in untreated brain metastases from NSCLC will be assessed in a phase II trial (NCT02085070).
Various other anti–PD-L1 antibodies have shown activity in NSCLC. An ORR of 20% was reported in a phase I trial of the engineered human IgG1 anti–PD-L1 inhibitor atezolizumab (Roche, Basel, Switzerland) (26, 27). The responses were durable, with 6-month PFS of 45%, and almost all responders were progression-free after 1 year. PD-L1 expression had a stronger correlation with ORR than other immune checkpoints. One phase III and three phase II
trials of atezolizumab are currently ongoing in NSCLC; the phase II FIR and BIRCH trials are investigating the safety and efficacy of atezolizumab in patients with PD-L1-positive tumors (NCT01846416), while the phase III OAK and phase II POPULAR trials are investigating atezolizumab in comparison with docetaxel as second-line treatment (NCT01903993) (37). Interim results from the POPULAR trial showed a significant OS benefit for atezolizumab (11.4 versus 9.5 months, p<0.05) (38). However, no activity in PD-L1 negative patients was noted. Efficacy of combinatorial approaches with erlotinib (NCT02013219), bevacizumab (NCT01633970) and the MEK inhibitor cobimetinib (NCT01988896) are evaluated in phase I trials.

Durvalumab (AstraZeneca) is an engineered human IgG1 monoclonal antibody that blocks PD-L1 binding to PD-1. Durvalumab has also shown activity in NSCLC. Interim results from the NSCLC cohort of an ongoing phase I study (n=18) in advanced solid tumors (NCT01693562) demonstrated early and durable activity in NSCLC with an ORR of 13% at 12 weeks. Durvalumab was well-tolerated, with no treatment discontinuations, drug-related colitis, or grade 3/4 pulmonary toxicities (28). Expansion cohorts are currently being enrolled for this trial to further test the safety and efficacy of durvalumab in advanced solid tumors, including NSCLC. Durvalumab will also be part of the multi-drug biomarker-driven phase II/III Lung-MAP trial that will assess several different drugs (durvalumab, rilotumumab, AZD4547, GDC-0032 or palbociclib) and combinations (anti-PD-L1 plus anti-CTLA-4) in NSCLC (NCT02154490). Furthermore, the phase II ATLANTIC study will assess the effects of durvalumab as third-line therapy in patients with advanced or metastatic NSCLC (NCT02087423).

The use of immune checkpoint blockade has mainly focused on the metastatic setting of NSCLC. However, there is growing interest in evaluating such therapies in earlier-stage NSCLC and extensive-stage small cell lung cancer. A phase III trial evaluating durvalumab as consolidation therapy in stage IIIB NSCLC after completing definitive chemoradiotherapy is already underway (PACIFIC trial; NCT02125461). Another trial evaluating adjuvant durvalumab after complete resection of stage IB, II, and III NSCLC is soon to open (BR31 IFCT1401 trial, NCT02273375).

**Combination Studies**

Novel approaches incorporating checkpoint inhibitors have shown promising preliminary results and durable responses in NSCLC and the search for optimal combination studies is emerging. There exists a strong rationale to add a further treatment modality to immuno-oncology based therapies. This approach includes combining two immuno-oncology agents that target different signalling pathways, as well as combining an immuno-oncology agent with chemotherapy, radiotherapy or a targeted-agent. For example, there exist several early- phase trials in patients with solid tumors investigating the potential of combining a CTLA-4 inhibitor with a PD-1 targeting-agent. Hypothetically, combining these two agents that target T-cell activation at different stages of the immune response will be a more potent anticancer treatment than therapy with each agent alone. CTLA-4 inhibitors (ipilimumab, tremelimumab) remove a physiological brake on T-cells, whereas anti-PD-1 antibodies remove a brake on activating during T-cell effector function. This combination may also overcome resistance to CTLA-4 blockade mediated by tumor PD-L1 expression or resistance to PD-1 blockade mediated by T-cell downregulation through the co-expression of CTLA-4 (12).

Preliminary results from an ongoing phase I trial (NCT01454102) of first-line ipilimumab and nivolumab in patients with advanced NSCLC (n=49) also highlight the promise of this approach. ORR was 13% in the nivolumab (1 mg/kg) plus ipilimumab (3 mg/kg) arm and 20% in the nivolumab (3 mg/kg) plus ipilimumab (1 mg/kg) arm; PFS at 24 weeks was 44% and 33% and 1-year OS was 65% and 44%, respectively. Combination therapy was associated with a safety profile that was manageable using well-established safety guidelines, and activity was observed in PD-L1-positive and PD-L1-negative patients suggesting that PD-L1 status may have no clear predictive value in patients with NSCLC treated with the combination of ipilimumab and nivolumab (39). The recommended combination dose for phase II/III trials shall yet to be determined.

An ongoing phase I trial is testing nivolumab monotherapy and nivolumab combinations with chemotherapy, targeted therapy and ipilimumab, as first-line treatments in chemotherapy-naïve NSCLC (CheckMate-012, NCT01454102). Preliminary data from the first 52 patients receiving nivolumab monotherapy in that study indicated a tolerable safety profile and an ORR of 21%; ORR was 31% in patients with PD-L1 positive and 10% in PD-L1 negative patients. Median PFS was 15.6 weeks and median OS was 98.3 weeks. Subgroup analysis suggested that response rates were higher in patients with a history of smoking (40).

Additional approaches to testing combined CTLA-4 and PD-1/PD-L1 pathway blockade, including the combination of tremelimumab and durvalumab, are also underway. A trial combining tremelimumab with durvalumab in NSCLC is ongoing (NCT02000947). Interim data from 61 patients indicate that the combination has a manageable safety profile with evidence of clinical activity, including in PD-L1-negative disease. These data support continued study of the combination; recruitment is ongoing (41).

A phase III study of durvalumab in monotherapy or in combination with tremelimumab *versus* standard-of-care in patients with advanced NSCLC who have received at least two prior systemic treatment regimes is currently

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A phase III study of durvalumab in monotherapy or in combination with tremelimumab *versus* standard-of-care in patients with advanced NSCLC who have received at least two prior systemic treatment regimes is currently
recruiting patients (ARCTIC trial, NCT02352948). Tremelimumab is also being evaluated in combination with gefitinib in NSCLC (NCT02040064) and durvalumab in advanced solid tumors and NSCLC (NCT01975831 and NCT02000947). The MYSTIC trial (NCT02453282), a phase III trial (durvalumab versus durvalumab plus tremelimumab versus platinum-based chemotherapy) for untreated NSCLC patients is also ongoing. The efficacy of pembrolizumab in combination with chemotherapy, targeted-agents, or ipilimumab will be determined in an ongoing phase I/II trial in stage IIIb/IV treatment-naive NSCLC (NCT02039674, KEYNOTE-021).

Another potential checkpoint combination therapy might include blockade of PD-1 and LAG-3, a molecule also involved in the regulation of T-cell activation. Combination therapy in mice has shown impressive activity (42). In addition, lirilumab (an anti-killer cell immunoglobulin-like receptor (KIR) antibody) is being assessed in combination with nivolumab or ipilimumab in patients with advanced solid tumors (NCT01714739, NCT01750580). Data are currently not available for these trials.

Identification of the specific immune-checkpoint pathway(s) that drive immune resistance is imperative to guide personalized therapeutic choices. There is preclinical evidence that oncogenes drive immune escape (e.g., B-raf inhibitors can increase tumor-infiltrating T-lymphocytes, and PD-L1 is upregulated by dabrafenib and trametinib) (43). In particular oncogenic EGFR signaling has been shown to remodel the tumor microenvironment by upregulation of the immunosuppressive molecules PD-1, PD-L1, and CTLA-4 (44). Blockade of oncogenic pathways might enhance or deter antitumor immunity, which provides the rationale for combination approaches involving targeted-agents and immunotherapy. Support for this proposal comes from a most recently published study by Spranger et al. (45). A molecular analysis of human metastatic melanoma samples revealed a correlation between activation of the WNT/β-catenin signaling pathway and absence of a T-cell gene expression signature. They identified the mechanism by which tumor-intrinsic active β-catenin signaling results in T-cell exclusion and resistance to anti-PD-L1/anti-CTLA-4 monoclonal antibody therapy. Specific oncogenic signals, therefore, can mediate cancer immune evasion and resistance to immunotherapies, pointing to new candidate targets for immune potentiation.

Gefitinib enhances cytotoxic activity of NK cells to human lung cancer cells. There have also been pre-clinical studies that demonstrate that EGFR inhibitors augment the expression of class I and class II MHC molecules and increase immune recognition by decreasing PD-1/PD-L1 expression (46). In addition, EGFR mutations upregulate immune checkpoint proteins PD-1 and PD-L1 in vitro and in vivo models of NSCLC (47). In a most recently published study immunohistochemistry revealed that PD-L1 and PD-1 were positive in 53.6% and 32.1% of mutation-positive NSCLC tumor specimens (cut-off threshold: 5%) (18). It has been shown that oncogenic EGFR mutations directly upregulate PD-L1 expression in NSCLC cell lines, and exposure to EGFR-TKIs (e.g., gefitinib) leads to PD-L1 downregulation (44), however this is not a consistent finding since other studies demonstrated no change of the PD-L1 status following TKI treatment (48). Moreover, anti-PD-1 antibodies significantly reduce tumor growth and prolong the survival of animals with EGFR-driven adenocarcinoma, events associated with marked increases in binding of the anti-PD-1 antibody to PD-1-expressing CD4+ and CD8+ T-cells and decreased levels of tumor-promoting cytokines (48). Consistently, it has been shown that PD-L1 positive NSCLC patients had a significantly longer OS (35.3 months) than those who were PD-L1 negative (19.8 months) in a cohort of patients with EGFR mutation-positive NSCLC treated with EGFR TKIs (18, 49). These findings raise the possibility that patients with EGFR-driven NSCLC might be particularly susceptible to PD-1 blockade immunotherapy. They also suggest a potential link between PD-L1/PD-1 expression and EGFR mutations in patients with NSCLC. In addition, for EGFR wild-type patients, PD-L1 overexpression was shown to be a poor prognostic indicator of OS (50).

Several early-phase trials combining targeted agents with immuno-oncology agents are therefore underway. Preliminary data from the cohort of CheckMate-012 with EGFR mutated advanced NSCLC receiving nivolumab and erlotinib suggest that this combination may provide durable clinical benefit coupled with an acceptable safety profile. OS at 18 months was 64% in patients receiving nivolumab and erlotinib, while ORR was 19%, median PFS was 29.4 weeks and the median duration of response was not reached (51).

However, one question that might also arise is whether EGFR-TKIs, currently the first-line treatment of EGFR mutation-positive NSCLC, might counteract anti-PD1/PD-L1 immunotherapy by downregulating its therapeutic target (i.e. PD-L1) in this tumor subtype. The answer to this question might soon come from multiple ongoing clinical trials evaluating the efficacy of anti-PD-1/PD-L1 antibodies combined with EGFR-TKIs in the treatment of patients with advanced NSCLC harbouring EGFR mutations, including nivolumab/erlotinib, pembrolizumab/erlotinib, or gefitinib (NCT02039674), durvalumab (anti-PD-L1) versus erlotinib (NCT02154490), and durvalumab/gefitinib (NCT02088112), and others. Most recently, the safety and tolerability results from a phase I study of durvalumab and gefitinib in NSCLC patients (NCT02088112) have been reported (52). The combination of durvalumab (10 mg/kg every two weeks) and gefitinib (250 mg/d) in TKI naïve NSCLC patients with EGFR mutations demonstrated an acceptable safety profile
and all patients in the dose-expansion phase (n=10) had shown tumor reductions.

The results published so far have demonstrated that PD-L1 is expressed in 30-40% of patients with EGFR mutation-positive lung adenocarcinoma, suggesting that at least a subset of the EGFR mutation-positive patients might be susceptible to PD-1/PD-L1 immunotherapy (44). The data have also indicated that PD-L1 expression significantly correlates with a greater disease control rate and longer PFS in response to EGFR-TKI therapy and prolonged OS in the cohort of patients with advanced lung adenocarcinoma with EGFR mutations.

These findings suggest that PD-L1 expression might represent a favourable biomarker for the therapeutic response to EGFR-TKIs and the prognosis of patients with EGFR mutation-positive NSCLC. Thus, the effects of PD-L1 expression on the clinical outcomes of patients with EGFR mutation-positive NSCLC who receive EGFR-TKI therapy warrant additional investigation.

Conclusion

The use of immunotherapy to unlock the immune system’s ability to eradicate cancer cells is an exciting new avenue for treatment of NSCLC, but the exact clinical applications are still not clear. Ongoing trials investigating combination immunotherapy with additional therapeutic agents, the impact of treatment sequence of immunotherapy with chemotherapy, and the possible role of maintenance therapy with checkpoint inhibitors should advance our knowledge and treatment options.

In NSCLC therapy, the emergence of targeted-agents with mechanisms of action based on driver mutations have introduced a set of standard predictive biomarkers to aid in clinical decision making. Immune checkpoint blockade, however, is designed to act on a complex and intact immunological pathway rather than individual mutations or antigens; as such, identification of a predictive biomarker has proven challenging (53). The most studied biomarker to date is PD-L1 expression, which has shown some utility for predicting response to agents targeting the PD-1 pathway in several but not all studies. The use of PD-L1 as a biomarker is complicated by a number of factors including the heterogeneity and dynamism of PD-L1 expression within tumors, variability in tissue collection timing, the antibody used for staining, definition of positivity, non-standardised test design, and the role of PD-L1 expression on tumor-infiltrating lymphocytes and other immune cells versus tumor cells. Moreover, for immune-based therapies, ORR may not be the optimal endpoint to assess the predictive role of biomarkers (54). In addition, it can be speculated that PD-L1 is not just “present” (positive) or “absent” (negative), since it is a biological continuum (Kerr 2015, personal communication). Consequently, findings from ongoing phase III trials should provide further information on PD-L1 and other biomarkers of response with immuno-oncology agents and lead to harmonisation of the assessment techniques used. Answers as to whether biomarkers such as PD-L1 can predict tumor responsiveness to agents targeting this pathway are still being equivocal so far.

Although anti–PD-1 and anti–PD-L1 therapies have shown encouraging activity with good tolerability in patients eligible for clinical trials, caution will need to be exercised when treating patients in clinical practice. The potential for severe immunemediated toxicity needs to be recognised, with clear algorithms in place to assist in rapid identification, evaluation, and treatment of such toxicities. Considering only roughly 20% of unselected patients treated with anti–PD-1/PD-L1 antibodies will have a meaningful response to therapy (47), other therapies will need to be pursued in most patients, and efforts to identify predictive biomarkers of response to this new class of drugs remain of importance.

The major goal of immunotherapy for patients with NSCLC is to prolong survival and quality of life by stimulating the patient’s own immune system to combat cancer. This new treatment approach, however, is still early in lung cancer, and more data from mechanistic and clinical studies are required on strategies to optimize the clinical impact of these therapies. Although PD-1/PD-L1 blockade therapy provides clinical benefits to approximately 20% of patients with advanced NSCLC, about 80% of patients still remain refractory to this treatment. Therefore, new molecules and combinations are urgently needed to address primary and secondary resistance to these new agents. Preliminary studies on tumor specimens collected from our patients will help to identify predictive immune biomarkers and synergistic combinations of anti–PD-1/PD-L1 monoclonal antibodies with standard therapies for NSCLC and other immunotherapies, and build the rationale for future clinical trials. In addition, it is still too early to know if gains in survival for NSCLC will be obtained with incremental approaches where we will just add anti-CTLA-4/anti–PD-1/PD-L1 monoclonal antibodies on top of existing NSCLC therapies.

In conclusion, we have not yet cut the Gordian helix–but as new treatment options for NSCLC are on the horizont we are making progress.

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Conflicts of Interest

W. Dempke and K. Edvardsen are employees of AstraZeneca Ltd. (UK). L. Sellmann and K. Fenchel declare not conflicts of interest.
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