Curr Oncol Rep (2016) 18: 59
DOI 10.1007/s11912-016-0544-7

Immune Checkpoint Blockade: A New Era for Non-Small Cell Lung Cancer

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Published online: 2 August 2016

Abstract Despite better understanding of it's molecular biology, non-small cell lung cancer (NSCLC) remains a challenging disease to treat. Unfortunately, treatment options are still very limited and prognosis for advanced disease is poor. Immune surveillance plays a crucial role in a host's defence against tumour cells, and this is particular relevant for lung cancer due to it's high somatic mutational load, which increases the chances for the immune system to recognize cancer cells as 'non-self'. Novel immunotherapies are emerging as an effective treatment for this disease. In this review, we present the data on immune checkpoint inhibitors for NSCLC, describing their mechanism of action, data efficacy from recent clinical trials, and strategies to select patients more likely to benefit from these agents.

Keywords Non-small cell lung cancer · Immune checkpoint · PD-1 · PD-L1 · CTLA-4 · Nivolumab · Pembrolizumab · Atezolizumab · Durvalumab

This article is part of the Topical Collection on *Translational Oncology*

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Introduction

Lung cancer is the first cause of cancer-related mortality in males and the second in females worldwide [1]. Non-small cell lung cancer (NSCLC) represents about 85 % of all new lung cancer diagnoses; it is often detected in advanced stages where treatment is palliative. Unfortunately, despite the improvement in clinical outcomes derived from new chemotherapeutics and targeted therapies inhibiting epidermal growth factor receptor (EGFR) mutations or anaplastic lymphoma kinase (ALK) gene rearrangements, the prognosis remains unfavourable [2]. In the last decade, immunotherapy treatments, including immune checkpoint inhibitors and cancer vaccines, have changed the treatment strategy for some types of cancer, particularly melanoma and more recently NSCLC [3]. In this review, we present the data on immune checkpoint blockade for NSCLC, describing the mechanism of action, data efficacy from recent clinical trials, and strategies to select patients more likely to benefit from these agents.

Immunoediting: Cancer and the Immune System

Cancer cells are able to escape attack from the immune system. The active interaction between the tumour and the immune system is described as "immunoediting" and is composed by three phases: elimination, equilibrium and escape.

The immune system is able to either stop or promote the cancer growth thus featuring evolving tumours, in different temporal moments. Paradoxically, the immune system is also able to eliminate tumour cells ab initio but, on the other hand, can help tumour progression by influencing tumour immunogenicity and maintaining chronic inflammation.

After the initial attempt by innate and adaptive immunity to eliminate totally or partially growing tumours, surviving

malignant cells will go into an equilibrium phase remaining quiescent until they are eventually able to escape the immune response [4]. This mechanism is further complicated by the progressive selection of more resistant tumour clones and by immunosuppressive action on the microenvironment, represented by a preferential recruitment of neutrophils and macrophages, with a concomitant exclusion of T lymphocytes, induced by tumour itself [5]. There is strong evidence that the composition of tumour immune infiltrates has a predictive and prognostic significance and that immune escape is one of the "Hallmarks of Cancer" [6].

The elimination phase is started by the appearance of new antigens representing the result of DNA damage. In particular, carcinogen stimuli are able to cause genomic alteration and, in case of failure of cellular DNA-repairing mechanisms, mutant or hyperploids tumour cells are able to proliferate; they express new antigens conjugated to major histocompatibility complex (MHC) class I, responsible for interaction with CD8+ cells, and NKG2D, for Natural killer (NK) cells and calreticulin. The immune response to cancer antigens is executed by CD8+ cells which induce apoptosis through the secretion of perforin and granzymes, after direct binding to MHC class I, FAS or TRAIL receptors on tumour cells or through dendritic cells intermediation to NK T cells, whose principal molecular effector is IFN-γ. Moreover, innate immunity contributes to cancer elimination with macrophages and granulocytes recruitment and activation (IL-12, IL-1, TNF-α, and ROS are their molecular effectors). A higher number of somatic mutations correlate with tumour immunogenicity and capacity for the immune system to recognize cancer cells as 'non-self' [7].

In the equilibrium phase, tumour cells not being destroyed in the elimination phase can remain in a state of functional dormancy controlled by the immune system.

In the escape phase some tumour cells undergo genetic and epigenetic changes and, due to constant immune pressure, tumour cell variants evolve and may escape immune surveillance by reducing immune recognition (by the absence of strong tumour antigens or loss of MHC class I, class I-like, or co-stimulatory molecules) and/or by increasing resistance or survival (such as increasing expression of STAT-3 or anti-apoptotic molecule Bcl2) or by developing an immunosuppressive tumour microenvironment (cytokines such as VEGF, TGF-β; immunoregulatory molecules such as IDO, PD-1/PD-L1 [programmed cell death 1 receptor /programmed cell death ligand 1], Tim-3/ galectin-9, LAG-3). In this phase, the immune system fails to restrict tumour outgrowth, and tumour cells are able to become clinically relevant and metastasize.

The PD-1/PD-L1 pathway represents a critical T-cell-related resistance mechanism. PD-1 is significantly up-regulated on cancer-specific T cells, suggesting functional exhaustion of these cells, and PD-L1 is expressed by a variety of epithelial cancers and haematological malignancies, suggesting that these malignancies may use the PD-1/PD-L1 signalling pathway to attenuate or escape anti-tumour T-cell immunity and thus facilitate tumour progression. It has been shown that PD-L1 in tumour cells can induce resistance to T-cell-mediated killing and inhibit tumour cell apoptosis induced by antigen-specific T cells [8].

Immune Checkpoint Inhibitors

Anti-PD-1 Antibodies

Nivolumab (BMS-936558, Opdivo®) is a fully human IgG4 monoclonal antibody that binds and blocks PD-1 [9, 10••]. Nivolumab showed promising safety and efficacy in early phase clinical trials in patients with pre-treated NSCLC [11–13]. More recently, two randomised, open-label, phase 3 studies CheckMate 057 [14••] and CheckMate 017 [15••] evaluated nivolumab as second-line treatment in patients with advanced NSCLC. In CheckMate 057, 582 patients with recurrent or stage 3B/4 non-squamous NSCLC who had progressed either during or after platinum-based doublet chemotherapy were treated with either nivolumab (3 mg/kg every 2 weeks) or docetaxel (75 mg/m^2 every 3 weeks) until progressive disease or intolerable toxicity. Median overall survival (OS) was longer in the nivolumab arm (12.2 vs. 9.4 months; HR for death 0.73, 96 % CI 0.59–0.89, $P = 0.002$). In contrast, there was no difference in median progression-free survival (PFS) (2.3 vs. 4.2 months; HR for disease progression or death 0.92, 95 % CI 0.77–1.11, $P = 0.39$). The objective response rate (ORR) was 19 % with nivolumab versus 12 % with docetaxel ($P = 0.02$). The incidence of grade 3–4 adverse events (AEs) was lower in the nivolumab group compared to docetaxel (10 vs. 54 %, respectively) and most common any grade treatment-related AEs included fatigue (16 vs. 29 %), nausea (12 vs. 26 %), decreased appetite (10 vs. 16 %) and asthenia (10 vs. 18 %). The most frequent any grade immune-related AEs were rash (9 vs. 3 % nivolumab vs. docetaxel, respectively), pruritus (8 vs. 1 %), diarrhoea (8 vs. 23 %), hypothyroidism (7 vs. 0 %), transaminitis (3 vs. 1 %), infusion-related reaction (3 vs. 3 %), pneumonitis (3 vs. <1 %) and erythema (1 vs. 4 %). In total, 78 % of patients had quantifiable PD-L1 tumour-cell membrane expression using the Epitomics anti-PD-L1 antibody clone 28–8. Subgroup analysis suggested a strong predictive association between PD-L1 expression (subgroups predefined as ≥1 %, ≥5 % and ≥10 %) and all efficacy endpoints. In the PD-L1-negative group (<1 % expression), there was no difference in OS and PFS between the two arms. There was no significant difference in the incidence of AEs in subgroups of patients who expressed PD-L1 (<1 % [i.e., PD-L1-negative] vs. ≥1 % [i.e., PD-L1-positive]).

CheckMate 017 had a similar design but randomized 272 patients with stage 3B/4 squamous cell NSCLC to receive either nivolumab (3 mg/kg every 2 weeks) or docetaxel (75 mg/m^2 every 3 weeks). Nivolumab improved median OS (9.2 vs. 6.0 months; HR for death 0.59, 95 % CI 0.44–0.79, $P < 0.001$), PFS (3.5 vs. 2.8 months; HR for death or disease progression 0.62, 95 % CI 0.47–0.81, $P < 0.001$) and ORR (20 vs. 9 %, $P = 0.008$) compared to docetaxel. A total of 83 % of patients had quantifiable PD-L1 tumour cell membrane expression using the Epitomics anti-PD-L1 antibody clone 28–8. In contrast to CheckMate 057, there was no predictive association between PD-L1 expression and efficacy. A significantly lower number of patients had grade 3–4 treatment-related AEs following nivolumab versus docetaxel (7 vs. 55 %, respectively). The commonest any grade treatment-related AEs were fatigue (16 vs. 33 % nivolumab vs. docetaxel, respectively), decreased appetite (11 vs. 19 %), asthenia (10 vs. 14 %) and nausea (9 vs. 23 %). Treatment-related AEs (of any grade) with a potential immunological cause included diarrhoea (8 vs. 20 % nivolumab vs. docetaxel, respectively), pneumonitis (5 vs. 0 %), hypothyroidism (4 vs. 0 %), raised serum creatinine (3 vs. 2 %) and rash (4 vs. 6 %).

On the basis of the results from CheckMate 057 and 017 studies, nivolumab has been approved as second-line treatment for advanced NSCLC by the U.S. Food and Drug Administration and European Medicines Agency without need for testing PD-L1 expression.

The role of nivolumab as first-line therapy for advanced NSCLC remains unclear. In a phase 1 study, 52 treatment-naïve patients with advanced NSCLC, including PD-L1 positive (≥5 % tumour cells) and PD-L1 negative patients, received nivolumab (3 mg/kg every 2 weeks) until progressive disease or unacceptable toxicity [16]. Median OS and PFS were 22.6 months (range 0.2 to 30.1+ months) and 15.6 weeks (range 0.1+ to 121.6+ weeks). The incidence of grade 3–4 AEs was 19 % in total. CheckMate 026 (NCT02041533) is a phase 3, randomised, open-label study that has completed recruitment of patients with either recurrent or stage 4 treatment-naïve PD-L1 positive NSCLC. Patients have been randomised to receive either nivolumamb (3 mg/kg every 2 weeks) or standard first-line platinum-based chemotherapy. The primary outcome is PFS and data from this trial is not yet reported.

Pembrolizumab (MK-3475, Keytruda®) is a humanised IgG4 monoclonal antibody also directed against the PD-1. The large (n=495) phase 1 study KEYNOTE-001 established the safety for Pembroluzimab (2 or 10 mg/kg every 3 weeks or 10 mg/kg every 2 weeks) in previously treated and untreated patients with locally advanced or metastatic NSCLC [17]. Efficacy outcomes correlated with greater PD-L1 expression (1–49 % vs. ≥50 %).

More recently, the randomised, open-label, phase 2/3 study KEYNOTE-010 ($n = 1,034$) compared Pembroluzimab (2 or 10 mg/kg every 3 weeks) vs. docetaxel (75 mg/m^2 every 3 weeks) as second-line therapy for recurrent or stage 3B/4 NSCLC [18••]. In line with KEYNOTE-001 eligibility for enrolment included ≥1 % expression of PD-L1 on tumour biopsy using the MERCK anti-PD-L1 antibody clone 22C3. The median OS was 10.4 months (95 % CI 9.4–11.9) versus 12.7 months (95 % CI 10.0–17.3) versus 8.5 months (95 % CI 7.5–9.8) for Pembroluzimab 2 mg/kg, 10 mg/kg and docetaxel, respectively. The HR for death for both Pembroluzimab 2 and 10 mg/kg versus docetaxel 0.71, 95 % CI 0.58–0.88, $P = 0.0008$ and 0.61, 95 % CI 0.49–0.75, $P < 0.0001$, respectively. Overall survival was significantly longer in patients with PD-L1 expression ≥50 % (HR for overall survival for Pembroluzimab 2 and 10 mg/kg versus docetaxel was 0.54, 95 % CI 0.38–0.77, $P = 0.0002$ and 0.50, 95 % CI 0.36–0.70, $P < 0.0001$, respectively). The median PFS was 3.9 (95 % CI 3.1–.4.1) vs. 4.0 (95 % CI 2.7–4.3) vs. 4.0 (95 % CI 3.1–4.2) months for Pembroluzimab 2 mg/kg, 10 mg/kg and docetaxel, respectively. There was no significant difference in the median PFS for Pembroluzimab 2 and 10 mg/kg compared to docetaxel (HR for progressive disease or death 0.88, 95 % CI 0.74–1.05, $P = 0.07$ and 0.79, 95 % CI 0.66–0.94, $P = 0.004$, respectively). In patients with PD-L1 expression ≥50 %, PFS was significantly longer with Pembroluzimab 2 and 10 mg/kg compared to docetaxel (HR for progressive disease or death 0.59, 95 % CI = 0.44–0.78, $P = 0.0001$ and 0.59, 95 % CI = 0.45–0.78, $P < 0.0001$, respectively). Grade 3–5 AEs were reported in 13 and 16 % of patients receiving Pembroluzimab 2 and 10 mg/kg, respectively, compared to 35 % in the docetaxel group. The commonest grade 3–5 AEs included neutropenia (0 vs. 0 vs. 12 % in the 2 vs. 10 mg/kg Pembroluzimab vs. docetaxel groups, respectively), fatigue (1 vs. 2 vs. 4 %) and asthenia (<1 vs. 1 vs. 2 %). Grade 3–5 immune-related AEs occurred in 20 and 19 % (2 vs. 10 mg/kg Pembroluzimab, respectively). The commonest any grade potentially immune-mediated AEs were hypothyroidism (8 vs. 8 % in the 2 vs. 10 mg/kg Pembroluzimab, respectively), hyperthyroidism (4 vs. 6 %), pneumonitis (5 vs. 4 %), severe skin reactions (1 vs. 2 %) and colitis (1 vs. 1 %). KEYNOTE-010 established that Pembroluzimab was more effective than docetaxel as second-line therapy in patients with advanced NSCLC who have PD-L1 expression of ≥1 % in tumour cells, with improved efficacy outcomes in patients with PD-L1 expression ≥50 %.

Three randomised, open-label, phase 3 studies are currently evaluating Pembroluzimab (200 mg every 3 weeks) in patients with treatment-naïve advanced EGFR and/or ALK wild-type NSCLC including KEYNOTE-024 (NCT02142738),

KEYNOTE-042 (NCT02220894) and KEYNOTE-189 (NCT02578680). In these trials, Pembroluzimab is given either in conjunction with standard first-line therapy (KEYNOTE-189) or in comparison to (KEYNOTE-024, KEYNOTE-042). In addition, KEYNOTE-042 is enrolling only patients with PD-L1 expression ≥1 % (stratified into 'strong' [≥50 %] and 'weakly' [1–49 %] positive groups) whereas KEYNOTE-024 includes only patients with PD-L1 expression ≥50 %. Table 1 summarizes the result of studies with anti PD-1 antibodies.

Anti-PD-L1 Antibodies

Programmed cell death 1 receptor has two principal activator ligands (PD-L1 and PD-L2). PD-L1 and PD-L2 can be expressed constitutively by immune cells (IC) and tumour cells (TC) or they are expressed by tumour cells as an acquired immune resistance mechanism. PD-L1 blockade can enhance the anti-tumour immune response [19–21].

At present, many anti-PD-L1 humanized antibodies are under investigation in clinical trials. Atezolizumab (MPDL3280A), a human IgG1 monoclonal antibody was investigated in the phase 2 POPLAR study which randomized patients with advanced NSCLC between atezolizumab (1200 mg every 3 weeks) and docetaxel (75 mg/m^2 every 3 weeks) as second-line treatment [22]. The primary endpoint of the study was OS by PD-L1 expression levels in the intention-to-treat (ITT) population. PD-L1 was simultaneously evaluated in TC and IC. The evaluation of the samples scored 0, 1, 2 and 3 according to the intensity of the staining. Overall survival was longer in the atezolizumab group (12.6 vs. 9.7 months; HR 0.73, 95 % CI 0.53–0.99, $P = 0.04$). Increasing improvement in OS was associated with increasing PD-L1 expression (TC3 or IC3 HR 0.49 [0.22–1.07, $P = 0.068$], TC2/3 or IC2/3 HR 0.54 [0.33–0.89, $P = 0.014$], TC1/2/3 or IC1/2/3 HR 0.59 [0.40–0.85, $P = 0.005$]. There was no difference in OS in the TC0 and IC0 population (9.7 vs. 9.7 months; HR 1.04 [0.62–1.75, $P = 0.871$]). Also, PFS and ORR tended to be higher with increasing PD-L1 expression. Subgroup analysis suggested that unlike OS, improved PFS and ORR with atezolizumab were limited only to the TC3/IC3 group. Atezolizumab was better tolerated than docetaxel with 11 % (vs. 39 %) of patients experiencing grade 3–4 treatment-related AEs.

The single arm phase 2 BIRCH study enrolled 659 pretreated or treatment-naive advanced NSCLC patients with high PD-L1 expression (TC2/3 and/or IC2/3) to receive atezolizumab (1200mg every 3 weeks) [23]. The ORR (primary endpoint) was 19 vs. 17 % in treatment-naïve and pretreated patients, respectively. After a median follow-up of 8.8 months, none of the groups reached the median OS, confirming the observation that these patients have a high chance of responding to checkpoint inhibitors. Notably, atezolizumab is the only drug developed assessing PD-L1 expression on tumour cells and the immune infiltrate. The final results of the ongoing OAK study (NCT02008227), randomizing unselected patients to receive atezolizumab (1200mg every 3 weeks) over docetaxel (75 mg/m^2 every 3 weeks), will clarify the role of PD-L1 expression as a biomarker. The safety profile is in line with those of patients treated with anti-PD-L1 drugs. Only one (<1 %) patient in the atezolizumab group versus three (2 %) patients in the docetaxel group died from a treatment-related AE.

Several ongoing trials are investigating the role of atezolizumab in different therapeutic settings (NCT02409342, NCT02367781, NCT02367794). A phase 1b study evaluated atezolizumab combined with carboplatin and either paclitaxel, pemetrexed or weekly nab-paclitaxel in patients with chemonaive locally advanced or metastatic NSCLC (NCT01633970) [24]. Patients received atezolizumab 15 mg/kg every 3 weeks with standard chemotherapy dosing for 4–6

Table 1 Efficacy results from phase 2 and 3 randomised studies of anti-PD-1 antibodies versus docetaxel in the pre-treated population

Author	Histology	*N*	mPFS (mos)		HR, *P* value	mOS (mos)		HR, *P* value
Brahmer et al.	Squamous	272	Nivo 3.5	Doce 2.8	0.62, $P < 0.001$	Nivo 9.2	Doce 6.0	0.59, $P < 0.001$
Borghaei et al.	Non-squamous	582	Nivo 2.3	Doce 4.2	0.92, $P = 0.39$	Nivo 12.2	Doce 9.4	0.73, $P = 0.0015$
Herbst et al. (Pembroluzimab 2 mg/kg)	Squamous and Non-squamous	688[a]	Pembro 3.9	Doce 4.0	0.88, $P = 0.07$	Pembro 10.4	Doce 8.5	0.71, $P = 0.0008$
Herbst et al. (Pembroluzimab 10 mg/kg)		689[b]	Pembro 4.0	Doce –	0.79, $P = 0.004$	Pembro 12.7	Doce –	0.61, $P < 0.0001$

Key: *Doce* Docetaxel, *HR* Hazard ratio, *mOS* Median overall survival, *mos* Months, *mPFS* Median progression-free survival, *Nivo* Nivolumab, *Pembro* Pembroluzimab

[a] 345 patients in Pembroluzimab 2 mg/kg and 343 in docetaxel group

[b] 346 patients in Pembroluzimab 10 mg/kg and 343 in docetaxel group

cycles followed by MPDL3280A maintenance therapy until disease progression. The ORR across all arms was 67 %, and responses were seen in each arm independent of PD-L1 expression.

Durvalumab (MEDI4736) is an anti-PD-L1 compound developed by Medimmune. Rizvi et al. reported data from a phase 1/2 study evaluating 198 patients treated with durvalumab 10 mg/kg every 2 weeks until unacceptable toxicity, progressive disease or up to 1 year (NCT01693562) [25]. Patients who completed 1 year of therapy could be rechallenged upon progression. PD-L1 expression was determined with Roche Ventana PD-L1 antibody (SP263). The ORR was 14 % (23 % in PD-L1 positive and 5 % in PD-L1 negative patients) with a manageable safety profile. In a multicentre, non-randomised, phase 1b study (NCT02000947) Antonia et al. enrolled 102 immunotherapy-naive advanced or metastatic NSCLC patients. Patients were treated in the dose-escalation phase with durvalumab in combination with the anti-CTLA-4 antibody tremelimumab [26]. The primary endpoint was safety. Durvalumab 20 mg/kg every 4 weeks plus tremelimumab 1 mg/kg showed the best safety profile and was chosen for the ongoing phase 3 study MYSTIC (NCT02453282). PD-L1 status does not correlate with anti-tumour activity. In the durvalumab/tremelimumab 1 mg/kg cohort, ORR was 23 % (2/9 PD-L1 positive and 4/14 PD-L1-negative patients).

Several other trials investigating the safety and efficacy of durvalumab alone or in combination with other drugs in various settings are ongoing (NCT02352948, NCT02453282 and NCT02542293). This drug is also under investigation after concurrent chemoradiotherapy in stage 3B patients (PACIFIC trial) and as third-line therapy (ATLANTIC and ARTIC trials).

Avelumab (MSB0010718C), a fully human IgG1 anti-PD-L1 antibody, was investigated in a large phase 1 study (EMR 100070–001) [27]. In this study, 184 pretreated NSCLC patients were treated with avelumab 10 mg/kg every 2 weeks. Minimum follow-up was 6 months and PD-L1 was expressed on 86 % of patients (cut-off for positivity 1 % immunohistochemistry [IHC] DAKO). The ORR was 13.6 % with one complete response, PFS rate at 48 weeks was 18.1 % and median OS was 8.4 months. Avelumab seems to be more effective in PD-L1 positive patients (ORR 15.6 vs. 10.0 %; PFS 12.0 vs. 5.9 months; OS 8.9 vs. 4.6 months in PD-L1 positive vs. PD-L1 negative patients, respectively). Grade 3–4 AEs rate was 12 %, with two toxic deaths. Currently, a phase 3 study (EMR 100070–004) is on going with the aim to compare avelumab versus docetaxel in subjects with NSCLC whom progressed after a platinum-containing doublet chemotherapy. Table 2 summarizes the result of studies with anti-PD-L1 antibodies.

Anti-CTLA-4 Antibodies

Ipilimumab is a human IgG1 monoclonal antibody currently approved for the treatment of advanced (unresectable or metastatic) malignant melanoma. A phase 2 trial investigated ipilimumab (10 mg/kg administered on day 1 of a 3 week cycle) plus carboplatin/paclitaxel versus chemotherapy plus placebo, for a maximum of 6 cycles, as first-line therapy for advanced NSCLC [28]. In the two experimental arms, ipilimumab was given together with chemotherapy in either a phased schedule (2 cycles of chemotherapy plus placebo, then 4 cycles of chemotherapy plus ipilimumab) or a concurrent schedule (4 cycles of chemotherapy plus ipilimumab, then 2 cycles of chemotherapy plus placebo), and then given every 12 weeks until disease progression. The primary outcome of this trial, which randomized 204 patients, was the 'immune-related' PFS (irPFS) which was met only for the phased schedule with a median irPFS of 5.7 versus 4.6 months (control arm; HR 0.72, 95 % CI 0.50–1.06; $P = 0.05$). Median PFS was 5.1 versus 4.2 months, respectively (HR 0.69, 95 % CI 0.48–1.00, $P = 0.02$). A non-significant increase in OS was reported with the phased schedule (12.2 vs. 8.3 months; HR 0.87, 95 % CI 0.59–1.28, $P = 0.23$), while the best irORR was 32 and 18 % and ORR 32 and 14 %, respectively. The concurrent schedule did not significantly improve irPFS (5.5 vs. 4.6 months, HR 0.81, 95 % CI 0.55–1.17, $P = 0.13$), PFS (4.1 vs. 4.2 months, HR 0.88, 95 % CI 0.61–1.27, $P = 0.25$), OS (9.7 vs. 8.3 months, HR 0.99, 95 % CI 0.67–1.46, $P = 0.48$), irORR (21 vs. 18 %) or ORR (21 vs. 14 %) versus chemotherapy alone. The most common AEs were grade 1–2. The overall incidence of grade 3–4 immune-related AEs (mainly diarrhoea, rash, colitis, hypophysitis and hypopituitarism) was 6 % for the control arm, 20 % for the concurrent arm, and 15 % for the phased arm. None of baseline factors (age, sex, performance status, disease stage and histology) had an apparent impact on irPFS or PFS, with the exception of histology. In fact, patients with squamous NSCLC who were enrolled in the phased schedule, reported a longer median irPFS (HR 0.55, 95 % CI 0.27–1.12) and OS (HR 0.48, 95 % CI 0.22–1.03) than the control arm. There were no differences for the non-squamous histology, either for concurrent or phased schedules. A phase 2 study comparing ipilimumab plus carboplatin/paclitaxel vs. placebo plus carboplatin/paclitaxel is currently on going (NCT02279732).

In a phase 1 study conducted in Japan, ipilimumab, at the dose of 3 or 10 mg/kg, was administered in combination with carboplatin/paclitaxel with a phased schedule. The ipilimumab dose of 10 mg/kg was recommended for further studies showing a dose-limiting toxicity (DLT) in only one patient (grade 3 enterocolitis, grade 3 hyperbilirubinemia and grade 4 hyperlipasaemia). A total of 15 patients were enrolled, reporting an ORR of 40 % [29].

Tremelimumab is a fully human IgG2 monoclonal antibody with high affinity for CTLA-4. Tremelimumab was investigated as maintenance therapy in a phase 2 study in patients with advanced NSCLC progressing after at least 4 cycles of platinum-containing regimens. A total of 87 patients were

Table 2 Results of main studies investigating anti-PD-L1 as single-agent in advanced NSCLC

Author	Type of study	Line	Primary endpoint	Treatment	No. pts	ORR (%)	PFS (months)	OS (months)	Grade ≥ 3 toxicity (%)
Fehrenbacher	IIR	2	OS	Atezolizumab vs Docetaxel	144	15	2.7	12.6	11
					143	15	3.0	9.7	39
Besse	II	1	ORR	Atezolizumab	139	19	5.5	82 %[a]	11
		2			267	17	2.8	76 %[a]	
		≥3			253	17	2.8	71 %[a]	
Rizvi	I/II	≥1	ORR	Durvalumab	149	14	NR	NR	6
Gulley	I	2	ORR	Avelumab	184	12	11.6[b]	NR	12

No.pts Number of patients, *ORR* Objective response rate, *PFS* Progression-free survival, *OS* Overall survival, *IIR* Phase II randomized, *NR* Not reported

[a] 6-month OS

[b] weeks

randomized to receive tremelimumab administered at the dose of 15 mg/kg every 90 days versus placebo. The primary objective of the study was PFS at 3 months, which was 20.9 vs. 14.3 % for tremelimumab vs. placebo, respectively. The ORR in the tremelimumab arm was 4.8 % and incidence of grade 3–4 AEs, mainly diarrhoea and colitis, was 20.5 % [30].

Combination Strategies

Tumours exploit more than one mechanism to avoid immunodetection and therefore there is a strong rationale for combining immuno-oncology agents that act on different targets, such as anti–CTLA-4 plus anti–PD-1 agents. This approach has already led to the first interesting results in metastatic melanoma, and it could represent the future of the treatment of patients with advanced NSCLC [31].

The combination of ipilimumab, at a dose of 1 or 3 mg/kg, and nivolumab, at a dose of 1 or 3 mg/kg, was administered as first-line treatment to 148 patients with NSCLC [32]. The ORR was 13–39 % in the four treatment arms, regardless of the histology and expression of PD-L1, although a slight increased activity has been observed in patients with PD-L1 expression >1 %. Median PFS was between 4.9 and 10.6 months in the four treatment arms. Grade 3–4 AEs were observed in 28–35 % of patients and mainly consisted of pneumonia, endocrine disorders, skin rash and gastrointestinal disorders. Another phase 1 study (KEYNOTE-021), still ongoing, is evaluating the combination of ipilimumab, at a dose of 1 or 3 mg/kg, and pembrolizumab, at the doses of 2 or 10 mg/kg, in patients with NSCLC pretreated with <2 lines of therapy [33]. The primary outcome includes tolerability and DLT in the first 3 weeks of treatment. No DLTs were recorded in the first 17 patients treated in any dose level, with grade 2 diarrhoea and rash being the most frequent toxicity. In the 11 patients evaluable for response, the ORR was 55 %. The combination of durvalumab and tremelimumab is currently being investigated within a phase 1b study [26]. A total of 102 patients with pre-treated NSCLC received durvalumab at doses ranging between 3 and 20 mg/kg plus tremelimumab at the doses of 1, 3mg/kg or 10 mg/kg every 2 or 4 weeks. The most frequent grade 3–4 AEs were diarrhoea (11 %), colitis (9 %) and the increase of the lipase (8 %); 36 % of patients experienced a serious adverse event; there were three toxic deaths. In the 63 evaluable patients, the ORR was 17 %, but 23 % in those 26 patients who received the combination of durvalumab 10–20 mg/kg and tremelimumab 1 mg/kg. In this group, the ORR was 22 and 29 % in the 9 and 14 patients with PD-L1 expression ≥25 or <25 %, respectively. For this reason, durvalumab at the dose of 20 mg/kg and tremelimumab at 1 mg/kg every 4 weeks will be investigated further.

Considering the interesting results already reported, the expectations for immuno-oncology in NSCLC are high. We hope that these expectations are met and that the agents currently in development led to an improvement of outcomes in NSCLC management.

The Quest for a Biomarker

Second- or greater line studies with several different immune checkpoint inhibitors have shown ORR ranging between 10 and 20 % in unselected NSCLC patients. A relatively small number of patients may have better outcomes on these immunomodulatory therapies. Anti-PD-1 or anti-PD-L1 therapy interrupts the inhibitory effect that PD-1/PD-L1 binding has on the immune response, releasing effector T cells to kill tumour cells. This particular immune checkpoint is one of several possible inhibitory checkpoints, but there are also stimulatory checkpoints possibly in play, and, a variety of immune cells are involved in regulating and effecting the overall immune response to solid tumour cells (10). In the case of tobacco-

induced NSCLC with a high mutational load, there may be a heavy neo-antigen burden on tumour cells, and the host immune system may develop a specific immune response to these 'non-self' antigens to a variable extent. Thus, the immune response is an exceptionally complex mechanism, conditional on many and often dynamic factors. Therapeutic blockade of only one factor will inevitably lead to variable effects, a crucial factor when considering responses to these drugs.

There is no evidence that global measures of immune response such as blood lymphocyte subset counts or circulating interleukin levels have any ability to predict response to these drugs. While it is known that the presence of macrophages, dendritic cells or particular subsets of lymphocytes within the tumour has a prognostic effect, at least in the postoperative setting [34], and intuitively one would expect some of these factors would be related to response, there is similarly a dearth of data. As a probable surrogate test for the degree of cellular immune response ongoing in a tumour, there have been reports of an mRNA-based immune gene expression signature having predictive power for immune checkpoint blockade, but data are limited [35].

In NSCLC, the presence of a relatively high mutational burden, or evidence of particular mutation types (G>T) associated with tobacco carcinogenesis, a so-called smoking signature, has been shown to correlate with higher likelihood of response to pembrolizumab [36]. This makes sense, given the probable greater neoantigen burden and may explain why patients with a KRAS mutations enrolled in Checkmate 057 had better outcomes than patients with EGFR mutations [14••]. Smokers in general have been noted to have higher response rates than never-smokers [14••]. For general utility, none of these factors offers a promising biomarker strategy. Mutational load assessed by Rizvi et al. was based on a whole exome sequencing assessment of thousands of genes. It remains possible that a targeted panel of genes with a high frequency of mutation in smoking-related NSCLC may offer a useful approach but more data is required. Similarly, other factors which are associated with genomic instability and high mutational burden (microsatellite instability, mismatch repair gene function, polymerase E mutations) have been associated with better responses to anti-PD-1/PD-L1 therapy in other tumour types but data are limited in NSCLC.

By far the greatest amount of data exist for PD-L1 IHC as a biomarker for anti-PD-1/PD-L1 therapy [37, 38••]. PD-L1 protein is either the target of drugs such as atezolizumab, avelumab or durvalumab or is the key partner in the interaction blocked by anti-PD-1 agents such as nivolumab or pembroluzimab. High levels of expression of PD-L1 in tumours suggest this mechanism of immune avoidance is active and therefore a prime target for this therapeutic approach. Across the vast majority of published studies, there is a consistently higher ORR seen in patients with higher expression of PD-L1 by IHC. These higher ORRs are now being reflected in improvements in PFS and OS for pembrolizumab in NSCLC and nivolumab in non-squamous NSCLC when compared to docetaxel [14••, 22]. Only in squamous cell NSCLC patients in the Checkmate 017 trial was there no difference in ORR between cases considered PD-L1 IHC negative versus those scored positive [15••]. Although PD-L1 IHC positive cohorts demonstrate higher ORRs, much is made of the fairly consistent observation that some responses to these drugs are also seen, at lower frequency, in cohorts scored 'negative' for PD-L1. This, coupled with the modest ORRs in most PD-L1 'positive' groups, has led to PD-L1 being considered a poor biomarker. This is an unreasonable conclusion. The biology of the system and the nature of the therapeutic intervention are completely different from previously encountered biomarker-driven treatments in NSCLC, as described above. Immunomodulatory therapy is very different from blockade of an addictive oncogene tyrosine kinase. ORRs are much lower, in part because only one step in a complex immune response is being inhibited. PD-L1 expression is dynamic and it is feasible that the IHC score may not reflect the patient's disease at the time the therapy is given. It is also known that PD-L1 expression is heterogeneous so that the biopsy sample may not fairly reflect the patient's overall disease burden. Finally, and crucially, PD-L1 protein expression is a continuous variable, from zero, through some, to modest, and then high levels. Any threshold used to artificially define a 'positive' and 'negative' cohort where expression is above or below the set level will not separate patients into responders and non-responders. Patients are likely to have a variable probability of response related to PD-L1 expression. This is very different from the binary testing scenario and probability of response seen to EGFR or ALK TKIs. All of these factors conspire to increase the chance of a response in the so-called PD-L1 negative group and drive a lower probability of response in those patients in the 'positive' group if only just above the threshold. Consequently, the choice of threshold could be extremely important. Depending on the drug in question and the line of therapy, thresholds defining PD-L1 positivity vary from over 1, 25 or 50 % of tumour cells.

It seems very likely that PD-L1 IHC testing will be used for the selection of patients for all of the currently available drugs, at least for some indications. Implementation of this testing in clinical practice will be a challenge. We currently have at least five anti-PD-1/PD-L1 agents at various stages of development, each with

their own, specially developed PD-L1 IHC assay validated in their respective clinical trials. There is no evidence that an alternative assay based upon another anti-PD-L1 IHC clone, developed as a 'home brew' test: a so called laboratory developed test, will be adequately predictive. Furthermore, there is no evidence that any one of the trial-proven assays could be used to select for an alternative drug. Efforts are underway to address some of these questions but while it will be possible to judge the technical equivalence of assays, it will be much harder to develop clinical validation. Until we know better, it may be necessary, indeed advisable, to maintain the relationship between specific drugs and the assays validated in trials.

Conclusion

Immunotherapy represents the new frontier in the battle against cancer including NSCLC. Recent trials with immune checkpoint inhibitors have showed very interesting results in pre-treated NSCLC patients. The manageable safety profile, the potential for a sustained disease control, and efficacy across a broad spectrum of patients are potential benefits of immuno-oncology agents. Thus, a large amount of on-going trials is investigating these drugs in all stages of disease and lines of treatment of NSCLC both as single-agent and in combination with chemotherapy or targeted agents. Due to the complex mechanisms of interaction between the immune systems and the cancer cells, selection of patients most likely to benefit is proving difficult and at present the only predictive 'biomarker' for higher ORR and OS is expression of PD-L1.

Overall, the expectations for immuno-oncology are high and we hope that these expectations are met to improve treatment options for NSCLC patients.

Compliance with Ethical Standards

Conflict of Interest Raffaele Califano has received compensation from Bristol-Myers Squibb, Roche, MSD, and AstraZeneca for service as a consultant.

Keith Kerr has received compensation from Bristol-Myers Squibb, Roche, MSD, and AstraZeneca for service as a consultant.

Robert David Morgan declares that he has no conflict of interest.

Giuseppe Lo Russo declares that he has no conflict of interest.

Marina Garassino has received compensation (paid to her institution) from Bristol-Myers Squibb, MSD, AstraZeneca, and Eli Lilly for service as a consultant.

Floriana Morgillo declares that she has no conflict of interest.

Antonio Rossi declares that he has no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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