Review article
Targeting PD-1 and PD-L1 in Non-Small-Cell Lung Cancer

Authors:
Rajiv Kumar[1,2], Dearbhaile Collins[1], Saoirse Dolly[1], Fiona McDonald[1], Mary ER O'Brien[1], Timothy A Yap[1,2]
[1] Royal Marsden NHS Foundation Trust, London, United Kingdom
[2]The Institute of Cancer Research, London, United Kingdom

***Corresponding author:**
Dr Timothy A Yap
Clinician Scientist and Consultant Medical Oncologist
Drug Development Unit
Royal Marsden NHS Foundation Trust and The Institute of Cancer Research
Downs Road, London SM2 5PT, United Kingdom.
Tel: 44-20-8661-3539
Fax: 44-20-8642-7979
E-mail: timothy.yap@icr.ac.uk

Short title: PD-1/PD-L1 inhibitors in NSCLC

Keywords:
Non-small-cell lung cancer, PD-1, PD-L1, immune checkpoint inhibitors, chemotherapy, radiotherapy, small molecule inhibitors, CTLA-4 antagonists.

Target Journal	Current Problems in Cancer
Keywords	-
Abstract	150 words – unstructured.
Word Count	<5000 words
Number of Tables & Figures	6 in total
Number of References	<75 references
Reference Formatting	Current Problems in Cancer Style

Abbreviations
NSCLC – non-small cell lung cancer; PD-L1 – programmed death-ligand 1; PD-1 – programmed cell death protein 1; CLTA-4 – cytotoxic T-lymphocyte associated protein 4; CMC – complement mediated cytotoxicity; ADCC – antibody-dependent cell-mediated cytotoxicity; mab – monoclonal antibody; IHC – immunohistochemistry; OS – overall survival; PFS – progression free survival; HR – hazard ratio.

Acknowledgements
The authors acknowledge NHS funding to the NIHR Biomedical Research Centre at The Royal Marsden and the ICR.

Disclosures
TAY has received research support from AstraZeneca and Vertex, and travel support from Vertex and Merck, and has served on advisory boards of Pfizer and Bristol-Myers Squibb.

Abstract

The last decade has witnessed rapid advances in the discovery and development of immune checkpoint inhibitors in cancer medicine, particularly drugs targeting programmed cell death 1 (PD-1) and programmed cell death ligand-1 (PD-L1) in non-small cell lung cancer (NSCLC). The proven antitumor efficacy coupled with low rates of drug-related toxicities observed with these novel immunotherapeutics have led to the registration of multiple PD-1/PD-L1 inhibitors, such as nivolumab, pembrolizumab and atezolizumab, in second-line advanced NSCLC, while durvalumab is in late phase trial testing. Ongoing clinical research is now focused on the development of PD-1/PD-L1 inhibitor monotherapy in neo-adjuvant, adjuvant and first-line advanced NSCLC settings. There is also much interest in using these drugs as a therapeutic backbone for rational combinations with other treatment modalities, including cytotoxic chemotherapies, other immunotherapies such as cytotoxic T-lymphocyte-associated protein-4 (CTLA-4) antagonists, molecularly targeted agents and radiotherapy.

Introduction

Non-small cell lung cancer (NSCLC) is in general associated with tumor DNA damage and mutations induced by carcinogens in tobacco smoke [1], which should provide sufficient tumor antigens to illicit an immune response in NSCLC. Initial efforts undertaken as early as the 1970s with Bacille Calmette-Guerin (BCG), interferon and other vaccine strategies in NSCLC were ineffective [2]. The last decade has seen a rapid improvement in our understanding of the molecular mechanisms of tumor immunology, in particular the role of the immune synapse or immune checkpoints in the suppression of an antitumor immune response. This has led to the development of novel immunotherapeutics, including immune checkpoint inhibitors, which specifically target the programmed cell death 1 (PD-1) receptor, programmed cell death ligand-1 (PD-L1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) receptor, as well as novel vaccines and cellular therapies. The inhibitor ipilimumab (Bristol-Myers Squibb) was the first to gain Food and Drug Administration (FDA) registration in 2011 for the treatment of melanoma, while other immune checkpoint inhibitors have also now achieved regulatory approval in different tumor types, including NSCLC. In this review, we focus on the development and subsequent registration of PD-1 and PD-L1 inhibitors in second-line advanced NSCLC. We briefly discuss the development of PD-1/PD-L1 inhibitor monotherapy in neo-adjuvant, adjuvant and first-line advanced NSCLC settings. We then detail the development of these drugs as a therapeutic backbone for rational combinations with other treatment modalities, including cytotoxic chemotherapies, other immunotherapies such as cytotoxic T-lymphocyte-associated protein-4 (CTLA-4) antagonists, molecularly targeted agents and radiotherapy.

PD-1 is an inhibitory cell-surface receptor that is expressed on activated T-cells, B-cells, natural killer cells, monocytes and dendritic cells. The effector function of T-cells that express PD-1 in the tumor microenvironment is suppressed when PD-1 is coupled to the ligand PD-L1 (B7-H1) or PD-L2 (B7-DC) on tumor cells. Inhibition of the PD-1/PD-L1 immune checkpoint using monoclonal antibodies prevents the inhibition of effector T-cell function, permitting T-cells to maintain their tumor cell killing function **(Figure 1)** [3].

There are now several PD-1/PD-L1 inhibitors already approved in NSCLC or which are at different phases of drug development **(Table 1)**. The anti-PD-1 IgG4 monoclonal antibodies are able to bind C1q and activate the complement pathway. This is in contrast to PD-L1 inhibitors, which are IgG1 monoclonal antibodies. The Fc region of IgG1 is able to induce antibody dependent cell mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity. However, this ADCC function is generally engineered out of Fc regions, because when binding to the PD-1/PD-L1 axis, ADCC could potentially increase toxicity through the killing of immune cells expressing PD-1/PD-L1. Avelumab (Pfizer) is an example of one of the current PD-L1 antibodies, which have retained ADCC function .

PD-L1 expression using immunohistochemistry has been investigated as a predictive biomarker and appears to have some value in predicting response in some tumor subgroups, however, it does not appear to have prognostic value. It is currently unclear if PD-L1 expression is a dynamic biomarker, although similar levels of PD-L1 expression have been observed on fresh contemporary tumor biopsies compared to those at diagnosis. It does appear that the highest level of tumor PD-L1 expression (>50% tumor cells), which is observed in about 28% of NSCLC patients, has the greatest predictive value for patient benefit [4].

Second-line PD-1/PD-L1 inhibitor monotherapy studies

There are several PD-1/PD-L1 inhibitors currently in clinical development, with nivolumab (Bristol-Myers Squibb) and pembrolizumab (Merck) leading the way for the management of NSCLC. Nivolumab monotherapy has been registered as second-line therapy for patients with NSCLC of squamous cell histology, unrestricted for PD-L1 status. The approval for non-squamous histological subtypes will follow soon on the basis of the CHECKMATE-017 [5] and CHECKMATE-057 [6] trials, which compare nivolumab to docetaxel as standard of care, respectively. Pembrolizumab monotherapy is also registered as second-line therapy for all pathological subtypes of NSCLC that express some degree of PD-L1 based on data from the KEYNOTE-001 [7] and KEYNOTE-010 [4] trials. On a practical note, pembrolizumab is administered 3-weekly due to its longer half-life [8] whereas nivolumab is administered 2-weekly, due to its shorter half-life when dosed at ≤3mg/kg [9]. The magnitude of the benefit from nivolumab or pembrolizumab monotherapy would appear to be greater than what can be achieved with docetaxel plus nintedanib (LUME-LUNG 1 trial) [10] and docetaxel plus ramuciramab (REVEL trial) [11] and it is unlikely that trials addressing the newer combinations of docetaxel will be pursued.

Herein, the pivotal trials for both nivolumab and pembrolizumab are outlined. For squamous NSCLC, CHECKMATE-017 reported a median overall survival (OS) of 9.2 months for nivolumab versus 6.0 months for docetaxel (HR=0.59, *P*<0.001), and a 1-year OS of 42% versus 24%, respectively. There was an excellent overall response rate (ORR) with 20% for nivolumab versus 9% for docetaxel. The median progression free survival (PFS) was 3.5 months for nivolumab and 2.8 months for docetaxel (HR=0.62, *P*<0.001). The median duration of response (DOR) had not been reached for the nivolumab arm and was 8.4 months for docetaxel arm. It is interesting that the PFS difference of 0.7 months translates to an OS of 3.2 months, implying that there are patients who derive long-term benefit from nivolumab that is not evident from PFS [5].

The equivalent study in non-squamous NSCLC, CHECKMATE-057, also demonstrated a significant survival benefit, with a median OS of 12.2 months for nivolumab versus 9.4 months for docetaxel (HR=0.73, *P*=0.0015), and an ORR of 19% versus 12% (*P*=0.02), respectively. Contrary to this OS benefit, the median PFS was not significantly different (HR=0.92, *P*=0.39). The Kaplan-Meier curves for both OS and PFS crossover, with an initial faster decline in the curve of patients receiving nivolumab, which may be explained in part by PD-L1 expression. When patients were analysed according to PD-L1 expression, patients with any level of PD-L1 expression (1%, 5% or 10%) derived both PFS and OS benefit from nivolumab. However, those without PD-L1 expression had a PFS and OS benefit similar to docetaxel [6]. As seen with squamous NSCLC patients, the DOR was exceptional, with a median DOR of 17.2 months (range: 1.8–22.6+ months) for nivolumab compared with 5.6 months (range: 1.2+-15.2+ months) for docetaxel. Many of these responses were ongoing when the trial was reported. The optimal duration of treatment, for example whether 1 or 2 years of therapy is enough as opposed to continuing treatment until disease progression with rechallenge on relapse, will be the subject of future trials.

In both CHECKMATE-017 and CHECKMATE-057 trials, nivolumab appeared less toxic compared to docetaxel. Grade 3-4 treatment-related adverse events were reported in 7–10% of the patients on nivolumab compared with 54–55% of those on docetaxel [5, 6]. While this seems reassuring, chemotherapy adverse events tend to be predictable, while immunotherapy toxicity is proving to be less predictable and may be relatively more severe.

Pembrolizumab has also shown excellent activity in the second-line advanced NSCLC setting and was recently granted accelerated FDA drug approval based on results from the KEYNOTE-001 [7] and KEYNOTE-010 studies [4]. KEYNOTE-001, an expanded access phase 1 trial assessing multiple drug doses included 495 patients with squamous and non-squamous advanced NSCLC. Of the patients treated, 81% were treatment naïve or had received at least one prior line of treatment. After a median follow-up of 10.9 months, the median OS was 12.0 months for all patients, and 10.4 months in previously treated patients. The median PFS was 3.7 months for all patients, and 3.0 months in previously treated patients. ORR was 19.4% in all patients, and 18.0% in previously treated patients. The median DOR was again more impressive than the PFS at 12.5 months (range: 1.0–23.3 months). When stratified for PD-L1 expression **(Table 1)**, tumor membranous staining of ≥50% was predictive of better response, PFS and OS. Interestingly, the response rates in smokers were double compared with non-smokers and there was little difference between squamous and non-squamous subtypes [7].

KEYNOTE-010 was a head-to-head assessment of pembrolizumab (2 mg/kg and 10 mg/kg) versus docetaxel in the second-line treatment of non-squamous NSCLC. The median OS was again in favour of pembrolizumab (2 mg/kg: 10.4 months, HR=0.71, *P*=0.0008; and 10 mg/kg: 12.7 months, HR=0.61, *P*<0.0001) over docetaxel (8.5 months). The median PFS was 3.9-4.0 months, with no statistical difference across the arms. When stratified for PD-L1 expression, PD-L1 positive patients (≥50% membranous staining), the median OS was also in favour of pembrolizumab (2 mg/kg: 14.9 months, HR=0.54, *P*=0.0002; and 10 mg/kg: 17.3 months, HR=0.50, *P*<0.0001) over docetaxel (8.2 months). Likewise, for this patient population, the median PFS was in favour of pembrolizumab (2 mg/kg: 5.0 months, HR=0.59, *P*=0.0001; and 10 mg/kg: 5.2 months, HR=0.59, *P*<0.0001) over docetaxel (4.1 months). The response rate was ~30% in PD-L1 positive patients receiving pembrolizumab versus 8% receiving docetaxel, and in all patients, this was 18% versus 9%, respectively [4].

Severe toxicities (grade 3-5) occurred in 13-16% with pembrolizumab, in contrast to 35% in patients receiving docetaxel. Treatment was discontinued due to treatment-related adverse events in 4-5% receiving pembrolizumab compared with 10% on docetaxel. Treatment-related deaths occurred in three patients in the 2 mg/kg arm (2 with pneumonitis and 1 with pneumonia), 3 patients in the 10mg/kg arm (1 each with myocardial infarction, pneumonia and pneumonitis), and 5 patients on docetaxel (1 each with acute cardiac failure, dehydration, febrile neutropenia, interstitial lung disease and respiratory tract infection). Immune-related adverse events occurred in ~20% of patients receiving pembrolizumab, with the most common being hyperthyroidism (4-6%), hypothyroidism (8%) and pneumonitis (4-5%). The immune-related adverse events of grade 3-5 severity occurring in ≥1% of patients were pneumonitis (2%) and severe skin reactions (1-2%) [4].

The studies involving atezolizumab monotherapy include a phase 2 and a randomised phase 3 study. The POPLAR phase 2 study compared atezolizumab (1200 mg i.v. given 3-weekly) against docetaxel in patients with advanced NSCLC second or third line. The interim analysis showed no difference in OS, with a median OS of 11.4 months versus 9.5 months, respectively (HR=0.77, *P*=0.11). However, the subgroup analyses showed that the OS benefit was restricted to patients with PD-L1 expression (either tumor membranous staining and/or intercalated infiltrating lymphocytes; HR=0.63, *P*=0.024) [12]. Mature follow-up data are awaited. The OAK phase 3 study comparing atezolizumab with docetaxel second-line has completed accrual and results are awaited [13].

With the atezolizumab phase II trials including treatment naïve patients (FIR study [13]) and pre-treated patients (BIRCH study [14]), the ORR was 27% with response rates being highest in patients who were strongly PD-L1 positive (tumour cells ≥50 and immune cells ≥10%). First-line patients were also observed to have a better ORR (29%) than second-line (and beyond) patients (17% of patients without brain metastases and 23% of patients with brain metastases). Based on the results of these trials, the FDA granted atezolizumab accelerated approval for second-line NSCLC patients whose tumors were PD-L1 positive.

The PD-L1 inhibitor durvalumab (10 mg/kg i.v. 2-weekly) has been assessed in a phase 1/2 monotherapy study in both squamous (n=82) and non-squamous (n=116) NSCLC. This study showed an ORR of 14% and a disease control rate of 24% at 6 months, with squamous patients demonstrating a better ORR than non-squamous patients (21% versus 10%). Again, the responses were durable, and many were ongoing at the time of interim reporting. Durvalumab was safe and tolerable at this dose, with the treatment-related toxicities observed in 48% of patients, most frequently fatigue (14%), decreased appetite (9%) and nausea (8%). Grade 3-5 treatment-related adverse events were reported in 6% of patients [15]; although, mature data are awaited.

First-line monotherapy PD-1/PD-L1 inhibitor studies

In addition to the data in treatment-naïve patients generated from the phase I expanded cohorts, there are now multiple ongoing NSCLC-specific first-line monotherapy randomised phase 3 studies, comparing PD-1/PD-L1 inhibitors with current platinum-based combination chemotherapy regimens **(Table 2)**. CHECKMATE-063 is also exploring the role of nivolumab monotherapy in the third line setting in squamous NSCLC [ref]. The results from these large international studies are eagerly awaited.

Neo-adjuvant and adjuvant PD-1/PD-L1 inhibitor monotherapy studies

There are several neo- and adjuvant studies already underway leading on from the antitumor efficacy that has been demonstrated in the advanced NSCLC setting. This is probably the best situation to demonstrate that an immunotherapeutic agent can cure more patients. The current studies that are recruiting are outlined in **Table 3**.

PD-1/PD-L1 inhibitor and chemotherapy combination studies

First-line platinum-based doublet chemotherapy is the standard of care for patients with advanced NSCLC who do not harbor an actionable driver aberration. Response rates of ~30% and a median OS of 8–10 months are typical. In theory, combining these chemotherapies with novel immune checkpoint inhibitors targeting the PD-1/PD-L1 axis have the potential to achieve an initial brisk tumor response and long-term tumor control that may translate into a survival benefit.

Early data report excellent response rates ranging between 33-55% in CHECKMATE-012 [16], 30-58% in KEYNOTE-021[17, 18], and 67% with early phase atezolizumab studies [19-21]. The obvious concern with combining these therapies is combinatorial toxicities, with grade 3-4 adverse events observed in 27% (KEYNOTE-021) to 45% (CHECKMATE-012) of patients. A summary of the key trials of PD-1/PD-L1 inhibitors combined with first-line chemotherapy is shown in **Table 4**. While the response rates are certainly impressive with these combinations, the toxicities also appear to be increased. Mature follow-up data are required on safety and survival from these ongoing studies. There are two further studies addressing the critical

question of the optimal sequencing of both chemotherapeutic and immunotherapeutic agents within combinations (NCT02591615 and NCT02581943).

PD-1/PD-L1 and CTLA-4 inhibitor combination studies

CTLA-4 inhibitors have been extensively developed in melanoma [22-24], however, at present they do not have a role in the management of NSCLC. Furthermore, there is no reliable biomarker that predicts for response to CTLA-4 blockade. Despite this, *in vitro* data support the potential for synergism from the combination of CTLA-4 inhibition with PD-1/PD-L1 axis blockade. Toxicity is an obvious concern given the immune-related toxicities seen with CTLA-4 antagonists as monotherapy. The actively recruiting trials are summarised in **Tables 2 and 6**.

In the clinical arena, adverse events are higher, as expected with the combination of immunotherapy versus either drug alone. The phase I KEYNOTE 021 examined advanced NSCLC progressing after at least one line of prior treatment with a platinum-doublet regimen. It assessed the safety and tolerability of the combination of pembrolizumab (2 or 10 mg/kg i.v. 3 weekly) with ipilimumab (0.3, 1.0 or 3.0 mg/kg i.v. 3 weekly). Interim data analysis reported no dose limiting toxicities or dose modifications in 17 patients with advanced NSCLC. Ten patients (59%) experienced grade 3-4 drug-related toxicities, predominantly rash, diarrhea and vomiting but also chills, cough, anorexia, weight loss, depression, dysphonia, fatigue, myalgia, pruritus, and pyrexia. Antitumor responses were observed in the 11 evaluable patients for all cohorts on treatment for greater than six weeks at the time of analysis, including one complete response (9%) and five partial responses (45%) [18].

In chemotherapy-naïve patients with advanced NSCLC, nivolumab combined with ipilimumab is a further focus. Patients were treated with either nivolumab 3 mg/kg and ipilimumab 1 mg/kg three-weekly, or nivolumab 1 mg/kg and ipilimumab 3 mg/kg three-weekly. Combination therapy was given over four doses, followed by two-weekly maintenance nivolumab. Treatment-related adverse events were reported in 39 patients (85%), with 48% experiencing grade 3-4 toxicities, leading to treatment discontinuation in 16 patients. Treatment-related deaths were observed in three patients including respiratory failure, bronchopulmonary hemorrhage and toxic epidermal necrolysis. Responses occurred in all cohorts with an overall response rate of 22%, which did not appear to correlate with PD-L1 status. These interim data suggest that a combined nivolumab and ipilimumab immunotherapy regimen is feasible and demonstrates antitumor activity in both PD-L1 positive and PD-L1 negative patients [25].

The combination of durvalumab and tremelimumab has been assessed in pre-treated locally advanced or metastatic NSCLC in a phase I clinical trial [26], where the maximum tolerated dose has not been reached from 61 patients tested to date. Toxicities reported include grade 3 transaminitis and grade 4 increased lipase. The most frequent drug-related adverse events include fatigue (26%), diarrhea (21%), and increased amylase (13%). A third of patients had more than one grade 3-4 treatment-related toxicity, with the most frequent being diarrhea (8%) and colitis (7%). Increasing doses of tremelimumab with a constant dose of durvalumab were associated with greater severity and frequency of adverse events. A fifth of patients had drug-related adverse events leading to discontinuation with the most frequent being colitis (7%). All adverse events were manageable with standard supportive therapy, including steroids, except grade 4 myasthenia gravis and grade 5 polymyositis in 1 patient. Of 31 patients, 8 patients (26%) had partial response and 11 patients (35%) had stable disease. Interestingly, the partial responses occurred in

3 of 10 patients with PD-L1-negative tumors. These data support the continued study of these combinations and recruitment to these studies are ongoing.

PD-1/PD-L1 inhibitor and molecularly targeted agent combination studies

Despite several targeted agents approved for patients with NSCLC harboring driver aberrations (*EGFR* mutations and *ALK* gene rearrangements), combination regimens with drugs targeting the PD-1/PD-L1 axis appear promising since both *EGFR* mutations and *ALK* gene rearrangements upregulate PD-L1 expression via the MEK-ERK and PI3K-AKT signalling pathways *in vitro* {Ota, 2015 #94}. These data suggest that oncogenic drivers induce immune escape in NSCLC through PD-L1 upregulation {Garon, 2015 #14}. For example, PD-1 positivity has been linked with *KRAS* mutations, while PD-L1 positivity has been associated with *EGFR* mutations or *ALK* translocations [27]. Furthermore, activation of the EGFR pathway induces PD-L1 expression, partly to facilitate evasion of the host anti-tumor immune response [28, 29]. Patients with *EGFR* mutant NSCLC with high PD-L1 were more sensitive to gefitinib or erlotinib and had improved tumor response rates and time to disease progression [27]. Similarly, PD-L1 can be upregulated by activation of key oncogenic pathways, such as the PI3K-AKT and the RAS-RAF-MAPK signalling pathways [30, 31]. Thus, high mutational rates may contribute to increased immunogenicity, indirectly predicting sensitivity to checkpoint inhibitors [32]. The development of resistance to multikinase inhibitor monotherapy has been coupled with increased PD-L1 expression and associated MET positivity [33]. Conversely, the pharmacological blockade of the PD-1 pathway using EGFR tyrosine kinase inhibitors reduced PD-L1 expression with subsequent tumor reduction [29].

Small molecule inhibitors with high response rates can result in substantial tumor lysis, releasing tumor antigens that can be presented to tumor-specific T-cells. These newly activated T-cells likewise upregulate inhibitory checkpoints such as CTLA-4 and PD-1. When these EGFR and ALK inhibitors are used in combination, anti-tumor T-cell responses including memory T-cell responses are enhanced, thereby increasing the potential for more durable disease control. Moreover, targeted agents may directly kill tumor cells and this may focus the newly activated immune response upon tumor antigens rather than self-antigens expressed by normal tissues, potentially reducing the risk of treatment-related toxicities.

Preclinical and early phase clinical data (KETNOTE-001 which included a limited number of *EGFR* mutant patients who had exhausted all lines of prior treatment) would suggest that prior therapy with EGFR inhibitors is associated with a lack of response to PD-1 blockade versus treatment-naïve patients. Therefore, a combination strategy makes sense rather than PD-1/PD-L1 inhibitor monotherapy. There are several early phase clinical trials assessing the combination of PD-1/PD-L1 inhibitors with small molecule agents targeted against EGFR (e.g. KEYNOTE-021, **Table 4**), EML4-ALK (e.g. NCT02013219), MEK (e.g. NCT02143466) and MET (e.g. NCT02323126). Early data show that the combination of PD-1/PD-L1 and EGFR inhibitors appears safe and well tolerated, as suggested by the results from the combination of gefitinib and durvalumab, although antitumor efficacy data are still awaited.

Cancer cells with defects in DNA repair pathways themselves can potentially activate the immune system. Therefore, inhibitors of the DNA damage response (DDR) that increase replication stress and increase genomic instability should be effective in

combination with immunotherapy. Exploiting the DNA damaging effects of DNA repair inhibitors, such as PARP and ATR inhibitors, may thus result in the potentiation of immunotherapy [34-36].

PD-1/PD-L1 inhibitor and radiotherapy combination studies

The integration of radiotherapy with immunotherapy is conceptually interesting as radiation may act as an antigen-releasing agent. Available pre-clinical data and anecdotal reports suggest the potential for abscopal effects and robust clinical responses in metastatic NSCLC. Prospective trials are ongoing to explore the safety and tolerability of immunotherapy combined with radiotherapy. In advanced stage disease, studies are looking at high-dose and low-dose palliative radiotherapy to a thoracic lesion (PEAR trial), while other studies are looking at the safety of stereotactic radiotherapy **(Table 8)**. For patients with locally advanced disease, durvalumab is being tested as a maintenance therapy after chemoradiotherapy (PACIFIC), while pembrolizumab is currently being assessed in a trial as a radiosensitiser with chemoradiotherapy (PARIS). These studies are summarised in **Table 8**.

Conclusions

These are exciting times in the field of immuno-oncology. The advent of these novel immune checkpoint inhibitors follows cumulative scientific and clinical efforts over the past 30 years. Superior monotherapy activity has now been confirmed compared to docetaxel in the second-line NSCLC setting. Overall, the safety, tolerability and efficacy of the multiple agents targeting the PD-1/PD-L1 axis appear to be similar across both PD-1 and PD-L1 inhibitors, with less than 10% of patients experiencing grade 3-4 treatment-related toxicities. There are no obvious features that make one drug stand out from the others. The focus of immunotherapy drug development has now switched to combining PD-1/PD-L1 with different chemotherapies, other checkpoint inhibitors, molecularly targeted agents and radiotherapy, as well as the development of analytically validated and clinically qualified predictive biomarkers of response. These rational studies are expected to further alter the treatment paradigm of NSCLC and positively impact patient outcomes.

Figure 1: Mechanism of action of PD-1 and PD-L1 inhibitors

Antigens, in the form of peptides, are presented by the major histocompatability complex (MHC) on antigen presenting cells (APC) and tumor cells to the T-cell receptor (TCR). Following T-cell activation, programmed cell death protein 1 (PD-1) receptors are expressed on the T cell surface and inhibits immune responses by engaging its two ligands PD-L1 and PD-L2. Monoclonal antibody (MAb) blockade of PD-1 and PD-L1 can enhance tumor immunity. Additionally, PD-1 and PD-L1 can be induced on other immune cells such as T-regulatory (Treg) or mast cells.

Tables

Table 1: Immune checkpoint inhibitors in clinical development.

Drug Name	Other names during development	Trade name	Class of agent	PD-L1 Companion Diagnostic Test
PD-1 inhibitors				
Pembrolizumab	MK-3475, Lambrolizumab	Keytruda	Humanized, IgG4 isotype mab against PD-1.	**Test:** PD-L1 IHC 22C3 Dako. Membranous staining of PD-L1 on tumor cells using IHC.
Nivolumab	ONO-4538, BMS-936558, MDX1106	Opdivo	Fully humanized, IgG4 isotype mab against PD-1.	**Test:** PD-L1 IHC 28-8 Dako. Membranous staining of PD-L1 on tumor cells using IHC.
PD-L1 inhibitors				
Atezolizumab	MPDL3280A	-	Humanized, IgG1 isotype mab against PD-L1.	**Test:** SP142 clone – Roche in house. PD-L1 expression on tumor cells and/or infiltrating lymphocytes using IHC.
Durvalumab	MEDI4736	-	Fully Humanized, IgG1 isotype mab against PD-L1.	**Test:** SP263 clone. Membranous staining of PD-L1 on tumor cells using IHC.
Avelumab	MSB0010718C	-	Fully humanized, IgG1 isotype mab against PD-L1. May induce ADCC.	N/A
CTLA-4 antagonists				
Ipilimumab	MDX-010, MDX-101	Yervoy	IgG1 isotype mab against CTLA-4.	N/A
Tremelimumab	Ticilimumab, CP-675,206	-	Fully humanized, IgG2 mab against CTLA-4.	N/A
Abbreviations: PD-L1 – programmed death-ligand 1; PD-1 – programmed cell death protein 1; CLTA-4 – cytotoxic T-lymphocyte associated protein 4; CMC – complement mediated cytotoxicity; ADCC – antibody-dependent cell-mediated cytotoxicity; mab – monoclonal antibody; IHC – immunohistochemistry.				

Table 2: Open-label randomised phase 3 clinical trial for first line monotherapy studies with PD-1/PD-L1 inhibitors in advanced or metastatic NSCLC.

Trial Name	Drug Name	Population
CHECKMATE-026 (NCT02041533) [37]	Nivolumab *vs* Investigator choice of platinum based combination chemotherapy.	Enriched for PD-L1 positive patients.
KEYNOTE-024 (NCT02142738) [38]	Pembrolizumab *vs* Investigator choice of platinum based combination chemotherapy.	PD-L1 positive (≥50%)
KEYNOTE-042 (NCT02220894) [39]	Pembrolizumab *vs* Investigator choice of platinum based combination chemotherapy.	PD-L1 positive (1-49% *vs* ≥50%).
IMpower110 (NCT02409342) [40]	Atezolizumab *vs* Pemetrexed-Platinum doublet chemotherapy.	Patients with non-squamous histology.
IMpower111 (NCT02409355) [41]	Atezolizumab *vs* Gemcitabine-Platinum doublet chemotherapy.	Patients with squamous histology.
NEPTUNE (NCT02542293) [42]	Durvalumab + Tremelimumab *vs* Investigator choice of platinum based combination chemotherapy.	
MYSTIC (NCT02453282) [43]	Durvalumab +/- Tremelimumab *vs* Investigator choice of platinum based combination chemotherapy.	

Table 3: Open-label, randomised, phase 3, adjuvant and neoadjuvant clinical trials in early stage NSCLC.

¥ Double-blind

¶ Single-arm phase 2 clinical trial.

Trial Name	Drug Names	Population
ANVIL (NCT02595944) [44]	Nivolumab *vs* placebo after standard chemotherapy.	
KEYNOTE-091 (PEARLS) (NCT02504372) [45]	Pembrolizumab *vs* placebo after standard chemotherapy for resected NSCLC.	
Adjuvant Atezolizumab (NCT02486718) [46]	Atezolizumab *vs* best supportive care, after cisplatin-based standard chemotherapy.	
Adjuvant Durvalumab¥ (NCT02273375) [47]	Durvalumab *vs* placebo. Patients may have had adjuvant chemotherapy (neoadjuvant chemotherapy is not permitted).	
Adjuvant & Neoadjuvant Durvalumab ¶ (NCT02572843) [48]	Durvalumab following neoadjuvant chemotherapy with Cisplatin-Docetaxel.	Neo-adjuvant and adjuvant treatment for resectable stage IIIA (N2 disease) NSCLC

Table 4: Studies looking at the PD-1/PD-L1 inhibitors in combination with standard first line chemotherapy.

Trial Name	Drug Names	Population	Study Design
CHECKMATE-012 (NCT01454102) [16, 49, 50]	Nivolumab with either gemcitabine + cisplatin, or pemetrexed + cisplatin, with carboplatin + paclitaxel.	First-line advanced or metastatic NSCLC.	Phase 1 clinical trial.
CHECKMATE-227 (NCT02477826) [51]	Nivolumab *vs* nivolumab + platinum-based chemotherapy (Platinum-Gemcitabine for squamous histology, & Platinum-Pemetrexed for non-squamous histology) Nivolumab vs Nivolumab + Ipilimumab.	First-line advanced or metastatic NSCLC.	Open-label, phase 3, randomised controlled trial.
CHECKMATE-370 (NCT02574078) [52]	nivolumab monotherapy, nivolumab + bevacizumab maintenance, nivolumab + pemetrexed maintenance, nivolumab + carboplatin + nab-paclitaxel, nivolumab + carboplatin + pemetrexed, nivolumab + carboplatin + docetaxel, nivolumab + carboplatin + gemcitabine, nivolumab + paclitaxel, nivolumab + docetaxel, nivolumab + gemcitabine, nivolumab + pemetrexed, nivolumab + erlotinib, nivolumab + crizotinib.	First-line advanced or metastatic NSCLC.	Open-label, phase 3, randomised controlled trial.
KEYNOTE-021 (NCT02039674) [17, 18]	Pembrolizumab with either with carboplatin-paclitaxel +/- maintenance bevacizumab, carboplatin-pemetrexed, erlotinib, gefitinib, or ipilimumab.	First-line advanced or metastatic NSCLC.	Phase 1/2 clinical trial.
KEYNOTE-189 (NCT02578680) [53]	Platinum-Pemetrexed based combination chemotherapy +/- Pembolizumab	First-line metastatic non-squamous - NSCLC.	Open-label, phase 3, randomised controlled trial.
IMpower 130 (NCT02367781) [20]	Carboplatin - *Nab*-paclitaxel +/- Atezolizumab	First-line metastatic non-squamous - NSCLC.	Open-label, phase 3, randomised controlled trial.
IMpower 131 (NCT02367794) [21]	- Carboplatin - *Nab*-Paclitaxel + Atezolizumab - Carboplatin - Paclitaxel + Atezolizumab - Carboplatin - *Nab*-paclitaxel	First-line metastatic squamous - NSCLC.	Open-label, phase 3, randomised controlled trial.
IMpower 150 (NCT02366143) [19]	Carboplatin - Paclitaxel +/- Bevacizumab, with or without Atezolizumab.	First-line metastatic non-squamous - NSCLC.	Open-label, phase 3, randomised controlled trial.
MSD NCT02591615 [54]	Phase 2 study assessing the optimal sequence of therapy with pembrolizumab before or after standard of care chemotherapy in the first line management of patients with locally advanced or metastatic stage IV NSCLC.	Phase 2 study assessing the optimal sequence of administering pembrolizumab, either before or after standard of care chemotherapy. Patients with squamous histology will receive carboplatin-paclitaxel, and patients with non-squamous histology will receive carboplatin-pemetrexed.	

Comprehensive Cancer Center of Wake Forest University, North Carolina, USA. (NCT02581943) [55]	Phase 2 clinical trial assessing the immune response in patients receiving pembrolizumab with or without chemotherapy in recurrent or stage IIIB/IV NSCLC	Phase 2 clinical trial assessing the immune response following treatment with pembrolizumab with or without combination chemotherapy with low dose carboplatin-paclitaxel for recurrent or stage IIIB/IV NSCLC.	

Table 5: Summary of clinical trials assessing small molecular targeted therapies combined with PD-1/PD-L1 inhibitors in patients with advanced non-small-cell lung cancer

Name of study	Clinical setting	Treatment
MedImmune study with Astrazeneca. NCT02088112 [56]	Phase 1 dose escalation and dose expansion study looking at the safety of the combination of durvalumab and geftinib.	Phase 1 dose escalation of durvalumab & gefinitib in locally advanced or metastatic NSCLC, with an EGFR mutation, with dose expansion at the maximum tolerated dose.
CHECKMATE-012 NCT01454102 [16]	Phase 1 study looking at the safety and tolerability of combining nivolumab with multiple established treatments for NSCLC in patients with stage IIIB/IV disease.	Phase 1 clinical trial combining nivolumab with erlotinib, ipilimumamb, bevacizumab maintenance, cisplatin-gemcitabine, cisplatin-pemetrexed, carboplatin-paclitaxel or as monotherapy.
Novartis study NCT02323126 [57]	Phase 2 study looking at the efficacy and safety of combing nivolumab with a Met inhibitor and an EGFR inhibitor, in previously treated patients with NSCLC.	This phase 2 clinical trial combines nivolumab with either EGF816, an EGFR inhibitor, or INC280, a Met inhibitor. Patients must have a T790M mutation or have cMET positivity, respectively, to be considered.
KEYNOTE-021 NCT02039674 [17]	Phase 1 study combining pembrolizumab with multiple established treatments for NSCLC in patients with stage IIIB/IV disease. For patients diagnosed with initially early stage disease, adjuvant treatment must been >1 year ago, with no treatment for recurrent disease.	Phase 1 clinical trial combining pembrolizumab with erlotinib, gefitinib, ipilimumab, carboplatin-paclitaxel, maintenance bevacizumab, and carboplatin-pemetrexed.
Hoffmann-La Roche trial NCT02013219 [58]	Phase 1b trial looking at the safety and tolerability of combining atezolizumb with erlotinib and alectinib, in patients with stage IIIB or IV disease.	This phase 1b clinical trial will look at escalating doses of either erlotinib or alectinib with fixed dosing of atezolizumab to establish the safety and tolerability of the combination. The maximum tolerated dose will be studied further in dose expansion. For the erlotinib cohort, patients in the dose escalation phase do not require an EGFR mutation and can have had multiple lines of prior treatment, however during the dose expansion phase, patients are required to have EGFR mutation and must be treatment naïve. All patients in the alectinib arm must have an ALK gene rearrangement.
Astrazeneca NCT02143466 [59]	Phase 1b study combining AZD9291 (Osimetinib) with novel therapies in patients with T790M EGFR mutation in patients with disease progression after first line therapy.	This phase 1b clinical trial combines AZD9291 with durvalumab, AZD6094 or selumetinib, in patients with a T790M EGFR mutation. In this trial, both drug will be undergo dose escalation, looking at the safety, tolerability, and pharmacokinetics.
Astrazeneca study NCT02179671 [60]	Phase 1 study looking at the safety and tolerability of combining durvalumab with either gefinitib, AZD9291, or selumetinib + docetaxel, or first line immunetherapy with CTLA-4 antagonist, with switch to durvalumab on disease progression.	Immune-Modulated Study of Selected Small Molecules (Gefitinib, AZD9291, or Selumetinib + Docetaxel) or a 1st Immune-Mediated Therapy (IMT; Tremelimumab) With a Sequential Switch to a 2nd IMT (MEDI4736) in Patients With Locally Advanced or Metastatic Non-Small-Cell Lung Cancer

Table 6: Summary of clinical trials assessing CTLA-4 antagonists combined with PD-1/PD-L1 inhibitors in patients with advanced non-small-cell lung cancer. These are in addition to the above mentioned CHECKMATE-021, CHECKMATE-227, KEYNOTE-021, and durvalumab-tremelimumab (Antonia et al ASCO2015). NEPTUNE (Table 2) and MYSTIC studies (Table 2)

Name of study	Clinical setting	Treatment
D4190C00006 NCT02000947 [61]	Phase 1b assessing the safety and tolerability of the combination of MEDI4736 and tremelimumab in patients with advanced NSCLC	The initial dose escalation phase will determine the maximum tolerated dose of MEDI4736 and tremelimumab in combination. This will be followed by subsequent dose expansion to gain further safety data.
ARCTIC NCT02352948 [62]	Phase 3 randomised controlled trial to assess the efficacy of combination of MEDI4736 with Durvalumab compared with standard chemotherapy for patients with locally advanced or metastatic NSCLC.	Treatments received: - MEDI4736 monotherapy (PDL1 positive study & PDL1 negative study) - Single agent chemotherapy (incl erlotinib) (PDL1 positive study & PDL1 negative study) - MEDI4736 + Tremelimumab (PDL1 positive study & PDL1 negative study) - Tremelimumab monotherapy (PDL1 negative)

Table 7: Summary of clinical trials assessing radiotherapy combined with PD-1/PD-L1 inhibitors in patients with advanced non-small-cell lung cancer.

Name of study	Clinical setting	Treatment	Reference
PACIFIC NCT02125461 [63]	Phase 3 randomised controlled trial of patients with stage III unresectable NSCLC looking at maintenance treatment after combined chemoradiotherapy.	Patients with locally advanced, unresectable stage III NSCLC, who had not progressed after concurrent platinum-based chemoradiotherapy. Patients are randomised (2:1) to 12 months of maintenance therapy with either durvalumab or placebo.	
PARIS	Phase 3 randomised controlled trial of patients with stage III unresectable NSCLC looking concurrent and maintenance treatment with pembrolizumab.	Patients with locally advanced, unresectable stage III NSCLC, are treated with concurrent chemoradiotherapy using pembrolizumab, followed by 12 months of maintenance pembrolizumab.	Personal communication with Prof Corinne Faivre-Finn, The Christie NHS Foundation Trust.
PEAR NCT02587455 [64]	Phase 1 trial looking at the safety and tolerability of combining escalating doses of pembrolizumab with palliative thoracic radiotherapy.	Patients will be treated with escalating doses of pembrolizumab (100 mg, 150 mg, and 200 mg) in combination with palliative thoracic radiotherapy at 20 Gy in 5 fractions or 36 Gy in 12 fractions, in a standard 3+3 dose escalation design.	
PRIMING	Phase 1 trial looking at the safety and tolerability of combining escalating doses of pembrolizumab with a fixed volume of stereotactic ablative radiotherapy to the thoracic cavity.	Patients will be treated with escalating doses of pembrolizumab (100 mg and 200 mg) in combination with fixed volume of stereotactic ablative radiotherapy to the thoracic cavity at 54 Gy in 3 fractions, in a standard 3+3 dose escalation design.	Personal communication with Dr Fiona McDonald, Royal Marsden NHS Foundation Trust.
Combination study at MD Anderson Cancer Centre NCT02444741 [65]	Phase 1, looking a t the safety and tolerability of pembrolizumab and either conventional radiotherapy or SBRT, followed by phase 2.	Phase 1 dose escalation study starting at 100 mg daily. Patients will be given SBRT at 50 Gy in 4 fractions or conventional radiotherapy with 45 Gy in 15 fractions. Phase 2 will be a dose expansion study in stage IV patients only at the maximum tolerated dose defined in the phase 1 study.	
Yale SBRT study in melanoma and lung cancer. NCT02407171 [66][65][64][63][64][62][81][8181818181818184]	Phase 1/2 clinical trial looking to induce responses to MK3475 in patients with metastatic melanoma and metastatic NSCLC	Phase 1 dose escalation study looking at the safety and tolerability of varying doses of SBRT with fixed dosing of pembrolizumab at 200 mg iv 3-weekly. Phase 2 will be a dose expansion at the MTD defined in the phase 1 component of the study.	
Netherlands Cancer Research Institute NCT02492568 [67]	Randomised phase 2 clinical trial of MK3474 in patients with Stage IV NSCLC, treated with at least one line of prior chemotherapy.	Patients will be randomised to either pembrolizumab alone or receive pembrolizumab 1-2 weekly after SBRT (8 Gy in 3 fractions). Pembrolizumab will be given at 200 mg iv 3-weekly.	
University of California Study NCT02599454 [68]	Phase 1 dose escalation study of atezolizumab combined with SBRT in inoperable stage I NSCLC.	Phase dose escalation study of atezolizumab combined with SBRT dosed at 50 Gy in 5 fractions, followed by dose expansion in the defined maximum tolerated dose.	

	Combination	Name of study	Study type	Target malignancy	Investigational agent	Recruitment details
Atezolizumab MPDL3280A	Small molecule inhibitor	NCT02655822	Phase 1/1b	Advanced cancers including NSCLC	CPI-444 (small molecule targeting adenosine-A2A receptor on T lymphocytes) alone or with atezolizumab (PD-L1 inhibitor)	N=534 Start Jan 2016 Estimated completion Dec 2018
		NCT01988896	Phase 1b	Advanced solid tumors	Atezolizumab (Anti-PD-L1 Ab) and oral cobimetinib	N=151 Start date Dec 2013 Estimated completion Oct 2017
	Immunotherapy	NCT02304393	Phase 1b	Advanced solid tumors	MPDL3280A (anti-PD-L1) [1200mg IV q21] in combination with RO7009789 (CD40 agonist)	N=160 Start date Dec 2014 Estimated completion Dec 2017
		NCT02350673	Phase 1b	Advanced solid tumors (CEA positive)	MPDL3280A (Anti-PD-L1, atezolizumab) and RO6895882 (immunocytokine targets CEA)	N=75 Start date June 2015 Estimated completion Feb 2019
		NCT02543645	Phase 1/2	Advanced cancer including NSCLC	Varlilumab (CDX-1127, anti-CD27) [0.3mg/kg / 1mg/kg / 3mg/kg] and atezolizumab (MPDL3280A, anti-PD-L1) [1200mg q21]	N=55 Start date Oct 2015 Estimated completion June 2019
Durvalumab MEDI4736	Small molecule inhibitor	NCT02088112	Phase 1	EGFR mutant locally advanced or metastatic NSCLC	MEDI4736 (anti-PD-L1) with gefitinib	N=56 Start date March 2014 Estimated completion January 2019
		NCT02572687	Phase 1	Advanced gastrointestinal or thoracic malignancies including NSCLC	Ramucirumab (LY3009806) plus MEDI4736	N=114 Start Feb 2016 Estimated completion August 2017
		NCT02403271	Phase 1b/2	Relapsed or refractory solid tumors including NSCLC	Ibrutinib (BTK inhibitor) plus durvalumab (MEDI4736)	N=160 Start date March 2015 Estimated completion June 2019
		NCT02484404	Phase 1/2	Advanced solid tumors including lung cancer	MEDI4736 [3mg/kg or 10mg/kg q14] in combination with cediranib (anti-angiogenic) [15mg or 20mg or 30mg daily continuous] or olaparib (DNA repair inhibitor) [200mg or 300mg bd continuous]	N=323 Start date June 2016 Estimated completion Dec 2019
	Immunotherapy	NCT02503774	Phase 1	Advanced solid tumors	MEDI9447 alone or in combination with MEDI4736 (durvalumab)	N=100 Start date July 2015 Estimated completion January 2021
		NCT02118337	Phase 1	Advanced malignancies	MEDI0680 (AMP-514) and MEDI4736 in combination	N=196 Start date May 2014 Estimated completion Nov 2018
		NCT02261220	Phase 1	Advanced solid tumors	MEDI4736 (durvalumab) in combination with tremelimumab	N=393 Start date Oct 2014 Estimated completion March 2018
		NCT02000947	Phase 1b	Advanced NSCLC	MEDI4736 plus tremelimumab	N=418

		D4190C00006				Start date Oct 2013 Estimated completion June 2018
		NCT02352948 ARCTIC	Phase 3	Advanced or metastatic NSCLC	MEDI4736 mono vs SoC in PD-L1 positive tumors AND MEDI4736 with tremelimumab vs SoC in PD-L1 negative tumors	N=730 Start date Jan 2015 Estimated completion Nov 2017
		NCT02453282 MYSTIC	Phase 3	First line advanced or metastatic NSCLC (EGFR wt and ALK wt)	MEDI4736 with tremelimumab combination therapy AND MEDI4736 monotherapy versus platinum-based SoC	N=780 Start date July 2015 Estimated completion May 2018
		NCT02542293 NEPTUNE	Phase 3	First line advanced or metastatic NSCLC (EGFR wt and ALK wt)	MEDI4736 with tremelimumab combination therapy vs platinum-based SoC	N= 800 Start date Nov 2015 Estimated completion Oct 2018
Nivolumab	Immunotherapy	NCT01714739	Phase 1	Advanced solid tumors	Anti-KIR antibody (Lirilumab) in combination with anti-PD-1 (nivolumab)	N=162 Start Oct 2012 Estimated completion October 2017
		NCT02439450 DURGA Trial	Phase 1b/2	NSCLC	Viagenpumatucel-L (HS-110, vaccine from irradiated lung cancer cells genetically engineered to secrete gp96-Ig) plus multiple treatment regimens including immunotherapies in particular nivolumab, anti PD-1	N=100 Start date April 2015 Estimated completion Mar 2019
		NCT02639234	Phase 2	Advanced and metastatic NSCLC	Vigil 1x 107 cells by intradermal injection q14 (4 to 12 doses) + nivolumab 3mg/kg IVI over 60mins q14	N= 35 Start date March 2016 Estimated completion Feb 2017
		NCT02659059 Checkmate 568	Phase 2	First line metastatic NSCLC	Nivolumab plus ipilimumab in combination	N=170 Start date Feb 2016 Estimated completion Dec 2016
	Small molecule inhibitor	NCT02518958 PRIMETIME	Phase 1	Advanced solid tumors	Nivolumab plus RRx-001 (epigenetic agent)	N=45 Start date July 2015 Estimated completion Sept 2017
Avelumab	Immunotherapy	NCT02554812 JAVELIN Medley	Phase 1b/2	Advanced malignancies	Avelumab in combination with other immunotherapies Initially avelumab + PF05082566, novel fully humanized IgG2 mAb agonist of 4-1BB (CD137, TNFRSF9)	N=147 Start date Nov 2015 Estimated completion Dec 2017
Pembrolizumab MK3475	Small molecule inhibitor	NCT02452424	Phase 1/2a	Advanced solid tumors including NSCLC	PLX3397 (oral inhibitor of CSF1 receptor) and pembrolizumab combination	N=400 Start date June 2015 Estimated completion July 2019
		NCT02437136 SNDX-275-0601	Phase 1b/2	Advanced metastatic or recurrent NSCLC	Entinostat (orally available HDAC inhibitor) with pembrolizumab	N=175 Start date July 2015 Estimated completion Oct 2019

	Immunotherapy	NCT02475213	Phase 1	B7-H3 expressing advanced cancers including NSCLC	MGA271 (Fc-optimized humanized MoAb) in combination with pembrolizumab	N=75 Start date July 2015 Estimated completion August 2020

References

[1] Gibbons DL, Byers LA, Kurie JM. Smoking, p53 mutation, and lung cancer. Mol Cancer Res. 2014;12:3-13.
[2] Al-Moundhri M, O'Brien M, Souberbielle BE. Immunotherapy in lung cancer. British Journal of Cancer. 1998;78:282-8.
[3] Ohaegbulam KC, Assal A, Lazar-Molnar E, Yao Y, Zang X. Human cancer immunotherapy with antibodies to the PD-1 and PD-L1 pathway. Trends Mol Med. 2015;21:24-33.
[4] Herbst RS, Baas P, Kim DW, Felip E, Perez-Gracia JL, Han JY, et al. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. Lancet. 2016;387:1540-50.
[5] Brahmer J, Reckamp KL, Baas P, Crino L, Eberhardt WE, Poddubskaya E, et al. Nivolumab versus Docetaxel in Advanced Squamous-Cell Non-Small-Cell Lung Cancer. N Engl J Med. 2015;373:123-35.
[6] Borghaei H, Paz-Ares L, Horn L, Spigel DR, Steins M, Ready NE, et al. Nivolumab versus Docetaxel in Advanced Nonsquamous Non–Small-Cell Lung Cancer. New England Journal of Medicine. 2015;373:1627-39.
[7] Garon EB, Rizvi NA, Hui R, Leighl N, Balmanoukian AS, Eder JP, et al. Pembrolizumab for the treatment of non-small-cell lung cancer. N Engl J Med. 2015;372:2018-28.
[8] Patnaik A, Kang PS, Rasco D, Papadopoulos KP, Elassaiss-Schaap J, Beeram M, et al. Phase I Study of Pembrolizumab (MK-3475; Anti–PD-1 Monoclonal Antibody) in Patients with Advanced Solid Tumors. Clinical Cancer Research. 2015;21:4286-93.
[9] Brahmer JR, Drake CG, Wollner I, Powderly JD, Picus J, Sharfman WH, et al. Phase I Study of Single-Agent Anti–Programmed Death-1 (MDX-1106) in Refractory Solid Tumors: Safety, Clinical Activity, Pharmacodynamics, and Immunologic Correlates. Journal of Clinical Oncology. 2010;28:3167-75.
[10] Reck M, Kaiser R, Mellemgaard A, Douillard JY, Orlov S, Krzakowski M, et al. Docetaxel plus nintedanib versus docetaxel plus placebo in patients with previously treated non-small-cell lung cancer (LUME-Lung 1): a phase 3, double-blind, randomised controlled trial. Lancet Oncol. 2014;15:143-55.
[11] Garon EB, Ciuleanu TE, Arrieta O, Prabhash K, Syrigos KN, Goksel T, et al. Ramucirumab plus docetaxel versus placebo plus docetaxel for second-line treatment of stage IV non-small-cell lung cancer after disease progression on platinum-based therapy (REVEL): a multicentre, double-blind, randomised phase 3 trial. Lancet. 2014;384:665-73.
[12] Spira AI, Park K, Mazières J, Vansteenkiste JF, Rittmeyer A, Ballinger M, et al. Efficacy, safety and predictive biomarker results from a randomized phase II study comparing MPDL3280A vs docetaxel in 2L/3L NSCLC (POPLAR). American Society of Clinical Oncology. Chicago, IL, USA. : J Clin Oncol; 2015. p. Abstr 8010.
[13] Rizvi NA, Chow LQM, Dirix LY, Gettinger SN, Gordon MS, Kabbinavar FF, et al. Clinical trials of MPDL3280A (anti-PDL1) in patients (pts) with non-small cell lung cancer (NSCLC). American Society of Clinical Oncology Annual Meeting. Chicago, IL, USA: J Clin Onc; 2014. p. TPS8123.
[14] Besse B, Johnson M, Jänne PA, Garassino MC, Eberhardt WEE, Peters S, et al. Phase II, single-arm trial (BIRCH) of atezolizumab as first-line or subsequent therapy for locally advanced or metastatic PD-L1-selected non-small cell lung cancer (NSCLC). European Cancer Congress (ECC). Vienna, Austria2015. p. LBA 16.
[15] Rizvi NA, Brahmer JR, Ou S-HI, Segal NH, Khleif S, Hwu W-J, et al. Safety and clinical activity of MEDI4736, an anti-programmed cell death-ligand 1 (PD-L1) antibody, in patients with non-small cell lung cancer (NSCLC). American Society of Clinical Oncology. Chicago, IL, USA. : J Clin Oncol; 2015. p. Abstr 8032.
[16] Bristol-Myers S. Study of Nivolumab (BMS-936558) in Combination With Gemcitabine/Cisplatin, Pemetrexed/Cisplatin, Carboplatin/Paclitaxel, Bevacizumab Maintenance, Erlotinib, Ipilimumab or as Monotherapy in Subjects With Stage IIIB/IV Non-small Cell Lung Cancer (NSCLC) (CheckMate 012). 2017.
[17] Merck S, Dohme C. A Study of Pembrolizumab (MK-3475) in Combination With Chemotherapy or Immunotherapy in Participants With Lung Cancer (MK-3475-021/KEYNOTE-021). 2016.
[18] Patnaik A, Socinski MA, Gubens MA, Gandhi L, Stevenson J, Bachman RD, et al. Phase 1 study of pembrolizumab (pembro; MK-3475) plus ipilimumab (IPI) as second-line therapy for advanced non-small cell lung cancer (NSCLC): KEYNOTE-021 cohort D. American Society of Clinical Oncology. Chicago, IL, USA. : J Clin Oncol; 2015. p. Abstr 8011.
[19] Hoffmann-La R. A Phase III Study of MPDL3280A (Anti-PD-L1 Antibody) in Combination With Carboplatin + Paclitaxel With or Without Bevacizumab in Patients With Stage IV Non-squamous Non-small Cell Lung Cancer IMpower 150. 2017.
[20] Hoffmann-La R. A Phase III Study of MPDL3280A (Anti-PD-L1 Antibody) in Combination With Carboplatin + Nab-Paclitaxel in Patients With Non-Squamous Non-Small Cell Lung Cancer IMpower 130. 2018.
[21] Hoffmann-La R. A Phase III Study of MPDL3280A (Anti-PD-L1) in Combination With Carboplatin + Paclitaxel or Carboplatin + Nab-paclitaxel Compared With Carboplatin + Nab-paclitaxel in Patients With Stage IV Squamous Non-Small Cell Lung Cancer (NSCLC) IMpower 131. 2023.
[22] Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med. 2010;363:711-23.
[23] Wolchok JD, Neyns B, Linette G, Negrier S, Lutzky J, Thomas L, et al. Ipilimumab monotherapy in patients with pretreated advanced melanoma: a randomised, double-blind, multicentre, phase 2, dose-ranging study. Lancet Oncol. 2010;11:155-64.
[24] Wolchok JD, Weber JS, Hamid O, Lebbe C, Maio M, Schadendorf D, et al. Ipilimumab efficacy and safety in patients with advanced melanoma: a retrospective analysis of HLA subtype from four trials. Cancer Immun. 2010;10:9.
[25] Wolchok JD, Kluger H, Callahan MK, Postow MA, Rizvi NA, Lesokhin AM, et al. Nivolumab plus ipilimumab in advanced melanoma. N Engl J Med. 2013;369:122-33.
[26] Antonia S, Goldberg SB, Balmanoukian A, Chaft JE, Sanborn RE, Gupta A, et al. Safety and antitumour activity of durvalumab plus tremelimumab in non-small cell lung cancer: a multicentre, phase 1b study. Lancet Oncol. 2016;17:299-308.
[27] D'Incecco A, Andreozzi M, Ludovini V, Rossi E, Capodanno A, Landi L, et al. PD-1 and PD-L1 expression in molecularly selected non-small-cell lung cancer patients. Br J Cancer. 2015;112:95-102.
[28] Sumimoto H, Imabayashi F, Iwata T, Kawakami Y. The BRAF–MAPK signaling pathway is essential for cancer-immune evasion in human melanoma cells. The Journal of Experimental Medicine. 2006;203:1651-6.
[29] Akbay EA, Koyama S, Carretero J, Altabef A, Tchaicha JH, Christensen CL, et al. Activation of the PD-1 pathway contributes to immune escape in EGFR-driven lung tumors. Cancer Discov. 2013;3:1355-63.
[30] Atefi M, Avramis E, Lassen A, Wong DJL, Robert L, Foulad D, et al. Effects of MAPK and PI3K Pathways on PD-L1 Expression in Melanoma. Clinical Cancer Research. 2014;20:3446-57.
[31] Parsa AT, Waldron JS, Panner A, Crane CA, Parney IF, Barry JJ, et al. Loss of tumor suppressor PTEN function increases B7-H1 expression and immunoresistance in glioma. Nat Med. 2007;13:84-8.
[32] Chen DS, Irving BA, Hodi FS. Molecular Pathways: Next-Generation Immunotherapy—Inhibiting Programmed Death-Ligand 1 and Programmed Death-1. Clinical Cancer Research. 2012;18:6580-7.

[33] Han JJ, Kim DW, Koh J, Keam B, Kim TM, Jeon YK, et al. Change in PD-L1 Expression After Acquiring Resistance to Gefitinib in EGFR-Mutant Non-Small-Cell Lung Cancer. Clin Lung Cancer. 2015.
[34] Postow MA, Callahan MK, Barker CA, Yamada Y, Yuan J, Kitano S, et al. Immunologic correlates of the abscopal effect in a patient with melanoma. N Engl J Med. 2012;366:925-31.
[35] Golden EB, Demaria S, Schiff PB, Chachoua A, Formenti SC. An abscopal response to radiation and ipilimumab in a patient with metastatic non-small cell lung cancer. Cancer Immunol Res. 2013;1:365-72.
[36] Grimaldi AM, Simeone E, Giannarelli D, Muto P, Falivene S, Borzillo V, et al. Abscopal effects of radiotherapy on advanced melanoma patients who progressed after ipilimumab immunotherapy. OncoImmunology. 2014;3:e28780.
[37] Bristol-Myers S, Ono Pharmaceutical Co L. An Open-Label, Randomized, Phase 3 Trial of Nivolumab Versus Investigator's Choice Chemotherapy as First-Line Therapy for Stage IV or Recurrent PD-L1+ Non-Small Cell Lung Cancer (CheckMate 026). 2016.
[38] Brahmer JR, Kim ES, Zhang J, Smith MM, Rangwala RA, O'Brien MER. KEYNOTE-024: Phase III trial of pembrolizumab (MK-3475) vs platinum-based chemotherapy as first-line therapy for patients with metastatic non-small cell lung cancer (NSCLC) that expresses programmed cell death ligand 1 (PD-L1). American Society of Clinical Oncology Annual Meeting. Chicago, IL, USA: J Clin Onc; 2015. p. TPS8103.
[39] Mok T, Wu Y-L, Watson PA, Zhang J, Rangwala RA. Phase 3 KEYNOTE-042 trial of pembrolizumab (MK-3475) versus platinum doublet chemotherapy in treatment-naive patients (pts) with PD-L1–positive advanced non-small cell lung cancer (NSCLC). American Society of Clinical Oncology Annual Meeting. Chicago, IL, USA: J Clin Onc; 2015. p. TPS8105.
[40] Hoffmann-La R. A Comparative Study of MPDL3280A (Anti-PDL1 Antibody) With Cisplatin or Carboplatin + Pemetrexed in Patients With Stage IV Non-Squamous Non-Small Cell Lung Cancer (NSCLC) IMpower 110. 2018.
[41] Hoffmann-La R. A Study of Atezolizumab (MPDL3280A) Compared With Gemcitabine + Cisplatin or Carboplatin in Patients With Stage IV Squamous Non-Small Cell Lung Cancer IMpower111.
[42] AstraZeneca. Study of 1st Line Therapy Study of MEDI4736 With Tremelimumab Versus SoC in Non Small-Cell Lung Cancer (NSCLC) (NEPTUNE). 2018.
[43] AstraZeneca. Phase III Open Label First Line Therapy Study of MEDI 4736 With or Without Tremelimumab Versus SOC in Non Small-Cell Lung Cancer (NSCLC). 2017.
[44] National Cancer I. Nivolumab After Surgery and Chemotherapy in Treating Patients With Stage IB-IIIA Non-small Cell Lung Cancer. 2018.
[45] Merck S, Dohme C, Etop, European Organisation for R, Treatment of Cancer E. Study of Pembrolizumab (MK-3475) vs Placebo for Participants With Non-small Cell Lung Cancer After Resection With or Without Standard Adjuvant Therapy (MK-3475-091/KEYNOTE-091). 2024.
[46] Hoffmann-La R. Study to Assess Safety and Efficacy of Atezolizumab (MPDL3280A) Compared to Best Supportive Care Following Chemotherapy in Patients With Lung Cancer. 2025.
[47] Group NCT, Intergroupe Francophone de Cancerologie T, Australasian Lung Cancer Trials G, National H, Medical Research Council A, National Cancer Institute N, et al. Double Blind Placebo Controlled Controlled Study of Adjuvant MEDI4736 In Completely Resected NSCLC. 2025.
[48] Swiss Group for Clinical Cancer R. Anti-PD-L1 in Stage IIIA(N2) NSCLC. 2021.
[49] Antonia SJ, Gettinger SN, Chow LQM, Juergens RA, Borghaei H, Shen Y, et al. Nivolumab (anti-PD-1; BMS-936558, ONO-4538) and ipilimumab in first-line NSCLC: Interim phase I results. American Society of Clinical Oncology. Chicago, IL, USA. : J Clin Oncol; 2014. p. Abstr 8023.
[50] Gettinger SN, Hellmann MD, Shepherd FA, Antonia SJ, Brahmer JR, Chow LQM, et al. First-line monotherapy with nivolumab (NIVO; anti-programmed death-1 [PD-1]) in advanced non-small cell lung cancer (NSCLC): Safety, efficacy and correlation of outcomes with PD-1 ligand (PD-L1) expression. American Society of Clinical Oncology Annual Meeting. Chicago, IL, USA: J Clin Onc; 2014. p. 8024.
[51] Bristol-Myers S, Ono Pharmaceutical Co L. An Open-Label, Trial of Nivolumab, or Nivolumab Plus Ipilimumab, or Nivolumab Plus Platinum-doublet Chemotherapy Versus Platinum Doublet Chemotherapy in Subjects With Stage IV Non-Small Cell Lung Cancer (NSCLC). 2018.
[52] Bristol-Myers S. A Master Protocol Study of Nivolumab in Advanced Non-Small Cell Lung Cancer (NSCLC) (CheckMate 370). 2019.
[53] Merck S, Dohme C. Study of Platinum+Pemetrexed Chemotherapy With or Without Pembrolizumab (MK-3475) in Participants With First Line Metastatic Non-squamous Non-small Cell Lung Cancer (MK-3475-189/KEYNOTE-189). 2017.
[54] Alliance Foundation Trials LLC, Merck S, Dohme C. Optimal Sequencing of Pembrolizumab (MK-3475) and Standard Platinum-based Chemotherapy in First-Line NSCLC. 2017.
[55] Comprehensive Cancer Center of Wake Forest U, National Cancer I. Effect of Pembrolizumab With or Without Carboplatin and Paclitaxel on Immune Response in Patients With Recurrent or Stage IIIB-IV Non-small Cell Lung Cancer. 2018.
[56] MedImmune LLC, AstraZeneca. MEDI4736 (Anti PD-L1) Combined With Gefitinib in Subjects With Non-Small Cell Lung Cancer(NSCLC). 2019.
[57] Novartis P, Novartis. Study of Efficacy and Safety of Nivolumab in Combination With EGF816 and of Nivolumab in Combination With INC280 in Patients With Previously Treated Non-small Cell Lung Cancer. 2017.
[58] Hoffmann-La R. A Phase 1b Study of Atezolizumab in Combination With Erlotinib or Alectinib in Participants With Non-Small Cell Lung Cancer. 2015.
[59] AstraZeneca. AZD9291 in Combination With Ascending Doses of Novel Therapeutics. 2017.
[60] AstraZeneca, Quintiles. Immune-Modulated Study of Selected Small Molecules (Gefitinib, AZD9291, or Selumetinib + Docetaxel) or a 1st Immune-Mediated Therapy (IMT; Tremelimumab) With a Sequential Switch to a 2nd IMT (MEDI4736) in Patients With Locally Advanced or Metastatic Non-Small-Cell Lung Cancer. 2016.
[61] MedImmune LLC. A Phase 1b Study of MEDI4736 in Combination With Tremelimumab in Subjects With Advanced Non-small Cell Lung Cancer. 2018.
[62] AstraZeneca. A Global Study to Assess the Effects of MEDI4736, Given as Monotherapy or in Combination With Tremelimumab Determined by PD-L1 Expression Versus Standard of Care in Patients With Locally Advanced or Metastatic Non Small Cell Lung Cancer. 2017.
[63] AstraZeneca. A Global Study to Assess the Effects of MEDI4736 Following Concurrent Chemoradiation in Patients With Stage III Unresectable Non-Small Cell Lung Cancer. 2017.
[64] Royal Marsden NHSFT, Merck S, Dohme C, Institute of Cancer Research UK, National Institute for Health Research UK. Pembrolizumab and Palliative Radiotherapy in Lung. 2018.
[65] Center MDAC, Merck S, Dohme C. MK-3475 and Hypofractionated Stereotactic Radiation Therapy in Patients With Non-Small Cell Lung Cancer (NSCLC). 2020.
[66] Evaluating the Combination of MK-3475 and Sterotactic Body Radiotherapy in Patients With Metastatic Melanoma or NSCLC.

[67] The Netherlands Cancer I, Merck S, Dohme C. Pembrolizumab After SBRT Versus Pembrolizumab Alone in Advanced NSCLC. 2016.
[68] University of California D, Genentech I. Atezolizumab and Stereotactic Body Radiation Therapy in Treating Patients With Non-small Cell Lung Cancer. 2017.