Chapter 3
Immunotherapy in Lung Cancer: A New Age in Cancer Treatment

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Abstract The management of Non-Small Cell Lung Cancer (NSCLC) has changed dramatically in the last 10 years with an increase in the understanding of the biology and with the development of new and multiple treatments. Chemotherapy being the first systemic treatment used in the setting of advanced disease, proving benefit for patients over palliative care. With the identification of oncogenic drivers, innovative targeted therapies were developed and tested, leading to important changes in the management of certain patients and giving to some of them the possibility to be treated in first line with oral inhibitors. Immunotherapy was then explored as a potential option, with promising results, and data of impact in important endpoints in lung cancer treatments. This chapter explores the different CTLA-4 inhibitors that have been investigated in NSCLC: ipilimumab and tremelimumab, as well as the different immune checkpoint inhibitors: anti PD-1 (nivolumab and pembrolizumab) and PD-L1 (atezolizumab, durvalumab,

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A. Naing, J. Hajjar (eds.), *Immunotherapy*, Advances in Experimental Medicine and Biology 995, https://doi.org/10.1007/978-3-030-02505-2_3

avelumab, BMS-936559) medications. It also analyzes the different studies that have been developed for NSCLC with these medications, the evidence obtained, and the possible role in the management of patients. Immunotherapy has definitely changed the paradigm on NSCLC treatment, and the future is promising for the benefit of patients.

Keywords NSCLC · Immunotherapy · PD-L1 and PD1 · Precision oncology · Pembrolizumab · Nivolumab · Atezolizumab · Immunotoxicity

Introduction

Non-small cell lung cancer (NSCLC) represents approximately 85% of all lung cancer cases. Most NSCLC patients are diagnosed with advanced stage disease and lung cancer is the leading cause of cancer-related death worldwide. Tobacco consumption is the most important risk factor associated with this disease and can account for regional differences in its epidemiology [1]. Environmental pollution and some mineral exposures are also associated with NSCLC; for example, some northern cities in Chile have a very high incidence of lung cancer and mortality due to lung cancer, which is thought to be related to arsenic concentrations in drinking water [2].

Until some years ago, metastatic NSCLC has been an incurable malignancy and only palliative treatments have been offered to patients with the purpose of improving quality of life and prolonging survival. In the late 1980s, a Canadian prospective randomized trial demonstrated that cisplatin-based chemotherapy combinations had a modest benefit in overall survival when compared with best supportive care in metastatic NSCLC patients; however, these treatments were associated with high toxicity [3]. Twenty years later, a meta-analysis showed a 9% benefit in 1-year overall survival in advanced NSCLC patients that received chemotherapy plus best supportive care compared with best supportive care alone [4]. The importance of histology in the treatment of advanced NSCLC patients has been highlighted by a randomized trial in which differences in overall survival were noted depending on the histologic subtype and type of cisplatin-based chemotherapy combination used [5].

After failure of first line of cytotoxic chemotherapy for metastatic NSCLC, docetaxel may be used as second-line treatment for patients with good performance status, with an overall survival benefit of 3 months when compared with best supportive care [6]. In patients with adenocarcinoma histology who were not treated with pemetrexed in the first-line setting, pemetrexed can be used as second-line therapy with similar overall survival outcomes when compared with docetaxel but with a significantly lower toxicity profile [7]. Patients whose disease progressed through second-line chemotherapy without significant worsening of their performance status can be considered for subsequent lines of treatment, but with unclear results and less literature to support it [8].

Adding an antiangiogenic drug to cytotoxic chemotherapy has become a strategy to improve survival in metastatic non-squamous NSCLC. Bevacizumab received approval by the FDA for this subset of patients based on the results of several clinical trials [9]. Nintedanib, an oral antiangiogenic drug that simultaneously inhibits VEGFR, FGFR,

PDGFR, and also RET [10], has received approval in Europe in combination with docetaxel for second-line metastatic non-squamous NSCLC patients. Among advanced lung adenocarcinoma patients, treatment with the combination of docetaxel plus nintedanib led to significantly improved median overall survival of 12.6 months, compared to 10.3 months with docetaxel plus placebo [1, 11].

Until the early 2000s, pathologic differentiation between NSCLC and small cell lung cancer was the main determining factor to guide oncologic treatment decisions. Among patients with NSCLC, it later became important to also distinguish between squamous and non-squamous histologies, with non-squamous comprised primarily of adenocarcinoma and large cell carcinoma subtypes. This classification allowed for appropriate chemotherapy regimens to be recommended for metastatic NSCLC patients based on histologic subtype. The subsequent discovery of new specific genetic and molecular alterations with potential targeted therapies found mainly in the non-squamous population led to further changes in treatment algorithms for advanced NSCLC. Mutations of the epidermal growth factor receptor (EGFR) and the echinoderm microtubule-associated protein-like 4-anaplastic lymphoma kinase (EML4-ALK) fusion gene [12] are the most clinically relevant given their epidemiologic frequency and the availability of targeted therapies. Other less frequent mutations in NSCLC include ROS-1, BRAF, HER2, MEK, MET, and RET.

Cancers are characterized by different genetic and epigenetic alterations. High rates of somatic mutations in lung cancer generate a variety of tumor-specific antigens and may contribute to increased immunogenicity [13]. Unfortunately, often oncogenic processes are studied independently of the antitumoral immune response (IR), which is a paradox, since one of the fundamental roles of the immune system (IS) is to distinguish self from foreign elements. Specifically, one factor which contributes to cancer development is the failure of various immunological mechanisms intended to eliminate altered antigens [14, 15]. With the aim of preventing the development of neoplasia, the immune system has different ways of recognizing cells that have escaped from the intrinsic suppressor mechanisms, identifying and destroying clones of transformed cells before they grow and form tumors, as well as recognizing and eliminating tumors already formed [16].

It is important to remember that the innate immune system is composed of dendritic cells, macrophages, natural killer (NK) cells, granulocytes (basophils, eosinophils, and neutrophils), complement proteins, chemokines and cytokines, among others. The innate immune system produces a rapid, nonspecific response to an antigen. In contrast, the adaptive IR, constituted by B lymphocytes, CD4 and CD8 T lymphocytes and antibodies, is a specific response toward a particular antigen which occurs more slowly, with the ability to leave immunological memory. The antitumor IR has been divided into seven stages [14–17], which make up the cancer-immunity cycle: (a) Release of cancer cells antigens (tumor cell death); (b) Cancer antigens presentation (fundamental role of dendritic antigen-presenting cells and professionals—APC); (c) APC and T cells priming and activation; (d) Trafficking of cytotoxic T cells to tumor; (e) T lymphocyte infiltration into the tumor (cytotoxic T lymphocytes, endothelial cells); (f) Recognition of tumor cells by T lymphocytes; and finally (g) Death of the tumor cells.

During the presentation phase, the APC presents the antigen to either T or B cells, which have a specific recognition receptor within their membrane (T cell receptor (TCR) or B cell receptor (BCR), respectively). However, this single signal is not sufficient to achieve lymphocyte activation and simultaneous presence of costimulatory molecules is required (interaction between CD80/CD28, CD40/CD40-ligand, CD86/CTLA-4, ICOS/ICOS ligand, among others). In addition, we must consider that every normal IR has mechanisms intended to prevent its perpetuation and the consequent damage associated with an exaggerated response. In this process, certain mechanisms are important: the participation of regulatory T cells (Tregs), the expression of inhibitory receptors (called checkpoints), the activation of apoptosis, and cell depletion [18].

Parallel to these events, tumors develop mechanisms to evade or to inhibit the IR, which include downregulation of antigen presentation (downregulation of the major histocompatibility complex—MHC), upregulation of inhibitors of apoptosis (Bcl-XL, FLIP), and expression of inhibitory cell surface molecules (programmed cell death ligand 1—PD-L1, FasL). In addition, tumor cells secrete factors that inhibit effector immune cell functions (TGF-β, IL-10, VEGF, LXR-L, IDO, gangliosides, or soluble MICA) or recruit regulatory cells to generate an immunosuppressive microenvironment (IL-4, IL-13, GM-CSF, IL-1β, VEGF, or PGE2). Once recruited, regulatory cells attenuate antitumor immunity through the liberation of immunosuppressive cytokines and by altering the nutrient content of the microenvironment. Specifically, secretion of IL-4 and IL-13 leads to recruitment and polarization of M2 macrophages, which express TGF-β, IL-10, and PDGF that inhibit T cells. The release of colony-stimulating factors IL-1β, VEGF, or PGE2 by tumor cells results in the accumulation of myeloid-derived suppressor cells (MDSCs) that can block T cell function by expressing TGF-β, ARG1, and iNOS. Tregs can also inhibit effector T cells through multiple mechanisms, including expression of CTLA-4 [16].

Based on these principles, immunotherapy was explored as a potential treatment option for malignancy. In NSCLC, initial vaccine trials failed to demonstrate benefit [2]. More recently, several immunotherapy agents have been developed which have proven beneficial in patients with NSCLC. These medications now have an established role in the management of NSCLC. Initial immunotherapy studies which evaluated agents that block the CTLA-4 pathway failed to show benefit in overall survival in NSCLC patients. However, anti-PD-1 and anti-PD-L1 treatment have shown impressive positive results for NSCLC patients when used as monotherapy, or in combination with other immunotherapy drugs or chemotherapy.

Pathways and Immunotherapy Drugs in NSCLC Treatment

CTLA-4 Pathway

The IS has counterregulatory mechanisms that limit potentially harmful amplification of the IR. Specifically, following antigen exposure, there is an upregulation of different molecules on the surface of the T cells, aimed at ending the IR. These

molecules are known as checkpoints, i.e., CTLA-4, LAG-3, PD-1/2, TIM-3. In some tumors, including lung cancer, the expression of these molecules is altered [19, 20]. CTLA-4 is constitutively expressed in Tregs, but only upregulated in conventional T cells after activation. It functions to inhibit the activation of these cells.

Once T cells are activated by the interaction between the MHC of the APC and the TCR, associated with costimulatory molecules (for example, CD28 binding to CD80/86), the CTLA-4 expression occurs at the level of the cell membrane. CD28 and CTLA-4 share identical ligands, CD80 and CD86. However, CTLA-4 has a higher overall affinity for both ligands. This interaction ends the IR. The critical role of CTLA-4 in maintaining self-tolerance is demonstrated by a rapidly lethal systemic immune-hyperactivation phenotype in knockout mice [21].

CTLA-4 was the first immune checkpoint targeted for cancer therapy in clinical practice. The anti-CTLA-4 antibodies interpose and prevent the interaction between CTLA-4 and its receptor, thereby inhibiting the completion of the IR and allowing the maintenance of the antitumoral IR. This is associated with the increase of the effector T cells and a dramatic reduction of the intratumoral Tregs [22, 23].

CTLA-4 Inhibitors

Ipilimumab

Currently, the most established CTLA-4 inhibitor is ipilimumab. This drug is a fully humanized IgG1 anti-cytotoxic T-lymphocyte antigen CTLA-4 monoclonal antibody that has the potential to block the binding of CTLA-4 to its ligand. By blocking the regulatory mechanisms of the T cell regulator CTLA-4, ipilimumab allows the immune system to attack the tumor cells [24].

First developed at the University of California, ipilimumab currently is under license of Bristol-Myers Squibb [25]. Ipilimumab was the first checkpoint inhibitor ever approved for cancer treatment. Hodi et al. published positive overall survival results in unresectable and metastatic melanoma patients when comparing ipilimumab with or without the combination of glycoprotein 100 peptide vaccine (gp100) against gp100 alone [26]. Despite the great favorable outcomes in unresectable or metastatic melanoma, NSCLC patients that have undergone treatment with ipilimumab monotherapy have not achieved the same positive results.

The assumption that tumor necrosis due to cytotoxic chemotherapy releases tumor antigens and may enhance the response to immunotherapy has been the basis of the rationale to combine carboplatin plus paclitaxel doublet chemotherapy with ipilimumab [27]. The interactions between ipilimumab and cytotoxic chemotherapy were tested by Weber in treatment-naïve melanoma patients in a phase I trial. Ipilimumab was given at a dose of 10 mg/kg intravenous every 3 weeks for a maximum of four doses; carboplatin was given at AUC of 6 and paclitaxel at 175 mg/m^2 every 3 weeks. Patients without limiting toxicity were allowed to receive maintenance ipilimumab starting at week 24 every 12 weeks until limiting toxicity or disease progression. No relevant pharmacodynamics or pharmacokinetics findings were found between both arms [28].

A phase 2 clinical trial that combined ipilimumab plus carboplatin/paclitaxel doublet chemotherapy was developed for chemotherapy-naïve stage IIIB/IV NSCLC patients whose disease was not amenable for curative treatment. The trial was a three-arm study (1:1:1) including 204 patients. The control arm was the doublet of carboplatin and paclitaxel for up to six cycles. Experimental arms included ipilimumab at a dose of 10 mg/kg given concurrently with the carboplatin/paclitaxel for four cycles followed by two doses of placebo; or two doses of placebo plus carboplatin/paclitaxel followed by ipilimumab plus the combination of carboplatin/paclitaxel for four cycles. Patients without limiting toxicity and/or without disease progression were allowed to receive ipilimumab/placebo treatment beyond the regular end of the treatment every 12 weeks as a maintenance therapy. Immune-related response criteria and modified WHO criteria were used to assess response. Immune-related progression-free survival (irPFS) was the primary endpoint of this trial; secondary endpoints were progression-free survival, overall survival, best overall response rate, immune-related best overall response rate and safety.

The primary endpoint, irPFS using immune-related RECIST criteria was met for the phased ipilimumab plus chemotherapy doublet (HR 0.72, $p = 0.05$) but not for the concurrent ipilimumab plus chemotherapy combination (HR 0.83, $p = 0.13$). Median irPFS was 4.6 months for the carboplatin plus paclitaxel combination, 5.5 months when adding concurrent ipilimumab, and up to 5.7 months when adding phased ipilimumab regimen. PFS using modified WHO criteria was also statistically significant in favor of the phased ipilimumab arm when compared with the control arm but not for the concurrent ipilimumab arm. Median overall survival was 8.3 months for the control arm and 12.2 months for the phased group (HR 0.87, $p = 0.23$); no overall survival advantage was reached in the concurrent ipilimumab group (9.7 months; HR 0.99, $p = 0.48$). The subgroup analysis showed a trend of benefit in irPFS and in overall survival in patients treated in the phased arm that had squamous histology when compared with non-squamous histology. Regarding toxicity, grade 3 and grade 4 adverse events were similar in the three arms: 37% in the control arm, 41% in the concurrent arm, and 39% in the phased arm. Hematological adverse events were similar in the ipilimumab-containing groups when compared with the carboplatin/paclitaxel group. Non-hematological, any grade (>15%) adverse events were most frequent in the control arm and included fatigue, alopecia, peripheral sensory neuropathy, nausea, and vomiting. Rash, diarrhea, and pruritus were higher in the ipilimumab groups than in the control arm. Immune-related grade 3–4 toxicities such as colitis, elevated transaminases and hypophysitis were higher in the ipilimumab-containing arms (20% for concurrent and 15% for phased ipilimumab groups) when compared with the control arm (6%). Two deaths related to treatment were reported, one of them was in the control group and the other in the concurrent group [29].

A phase III study was recently published evaluating the efficacy and safety of first-line ipilimumab or placebo plus paclitaxel and carboplatin in advanced squamous NSCLC. Patients with stage IV or recurrent chemotherapy-naïve squamous NSCLC were assigned (1:1) to receive paclitaxel and carboplatin plus ipilimumab 10 mg/kg or placebo every 3 weeks on an induction schedule comprised of six chemotherapy cycles, with ipilimumab or placebo from cycles 3 to 6 followed by

ipilimumab or placebo maintenance every 12 weeks for patients with stable disease or response. The primary endpoint was overall survival (OS). Nine hundred and fifty-six patients were included, with 749 received at least one dose therapy (chemotherapy plus ipilimumab, $n = 388$; chemotherapy plus placebo, $n = 361$). Median OS was 13.4 months for chemotherapy plus ipilimumab and 12.4 months for chemotherapy plus placebo (hazard ratio, 0.91; 95% CI, 0.77–1.07; $p = 0.25$) [3]. Another phase 1 clinical trial that combines either erlotinib or crizotinib, depending if patients have EGFR or ALK mutated status, plus ipilimumab is also currently ongoing (NCT01998126) [30]. Results from both trials will be very important to confirm the potential benefit of combining ipilimumab with cytotoxic chemotherapy in squamous NSCLC, or combining ipilimumab with target therapies in NSCLC patients that have an EGFR common mutation or an ALK translocation.

Ipilimumab in combination with other immunotherapy drugs will be discussed later in this chapter.

Tremelimumab

Tremelimumab is an anti-CTLA-4 IgG2 fully humanized monoclonal antibody [31]. Despite the similar mechanism of action than ipilimumab, tremelimumab as monotherapy has not shown benefit in NSCLC patients. In a phase 2 clinical trial for locally advanced or metastatic NSCLC patients with good performance status that had received four or more cycles of a platinum-based chemotherapy and had responded were randomized to tremelimumab or to best supportive care. The primary endpoint of the trial, progression-free survival, was not met, with an objective response rate of only 4% in the treated group. Grade 3–4 adverse events were reported in 20% of patients (including 9% of immune-related toxicities) versus none in the best supportive care arm [32].

Currently, a phase 1 clinical trial that studies tremelimumab plus gefitinib combination is ongoing for pretreated patients with stage IIIB and IV EGFR-mutated NSCLC (NCT02040064) [33].

Tremelimumab in combination with other immunotherapy drugs will be discussed ahead in this chapter.

PD-1/PD-L1 Pathway

The PD-1 receptor (Programmed Cell Death-1) is expressed in T/B cells, NK, and MDSCs after their activation. Its main function is to limit the activity of T cells in peripheral tissues, where the effector phase takes place (in contrast to the anti-CTLA-4 antibodies that fulfill their role in the initial activation of T cells). Excessive induction of PD-1 in the setting of a chronic antigenic exposure can induce anergy or exhaustion [19–35]. Inflammatory signals in tissues, mainly IFN-y, induce the expression of two ligands of this molecule, PD-L1 and PD-L2 (Programmed Cell

Death Ligand 1 and 2, respectively), which downregulates the activity of T cells, limiting collateral tissue damage and maintaining the self-tolerance.

Numerous tumor types express high PD-L1 levels, including NSCLC, suggesting that PD-1/PD-L1 pathway activation is a common mechanism used by tumors to avoid immune surveillance and growth [36, 37].

Specifically, the effects of PD-1/PD-L1 interaction include inhibition of T cell proliferation, survival and effector functions (cytokine release and cytotoxicity), and promotion of differentiation of CD4+ T cells into Tregs. PD-1 is expressed on a large proportion of tumor-infiltrating lymphocytes (TILs) which appear to be "exhausted," functionally inhibited, due to chronic antigen stimulation. This exhausted state was partially reversible by PD-1 pathway blockade in murine models of chronic viral infections [19].

Blockade of PD-1 signaling can restore CD8+ T cell functions and cytotoxic capabilities from the exhausted phenotype and enhance antitumor immunity, as demonstrated in preclinical studies [38, 39].

Anti-PD-1 Drugs

Nivolumab

Nivolumab (Opdivo®, Bristol Mayer Squibb) is a genetically engineered, fully human immunoglobulin G4 (IgG4) monoclonal antibody specific for human PD-1 [40].

The IgG4 isotype was engineered to obviate antibody-dependent cellular cytotoxicity (ADCC). An intact ADCC has the potential to deplete activated T cells and tumor-infiltrating lymphocytes and diminish activity as PD-1 is expressed on T effector cells and other immune cells. Nivolumab binds PD-1 with high affinity and blocks its interactions with both PD-L1 and PD-L2 [41].

In the CA 209-003 study, a phase 1 clinical trial that included patients with NSCLC, melanoma, castration-resistant prostatic cancer, renal cancer, and colorectal cancer, patients were enrolled to receive nivolumab at a dose of 0.1–10 mg/kg every 2 weeks to a maximum of 12 doses or until a complete response was achieved, limiting toxicity, progressive disease, or withdrawal of the consent for this trial. The primary objectives were to evaluate safety and tolerability. The trial was designed as a dose escalation and cohort expansion that included 122 NSCLC patients (47 squamous, 73 non-squamous, 2 unknown) from a total of 296 patients that were enrolled in the trial. Eighty-five percent of the NSCLC patients had received at least two prior lines of treatment including a 34% of patients receiving a tyrosine kinase inhibitor. The maximum tolerated dose for nivolumab was not reached. In the NSCLC expansion cohort, regardless of the histologic subtype, patients were randomized to nivolumab at doses of 1, 3, or 10 mg/kg. There were 11 deaths (4%) related to serious adverse events, none of which were secondary to nivolumab according to the investigators' reports. Fourteen NSCLC patients that underwent treatment had an objective response, 6% at dose of 1 mg/kg, 32% at dose of 3 mg/kg, and 18% at dose of 10 mg/kg. The global response rate for squamous and non-

squamous non-small cell lung cancer was 33% and 12%, respectively. Eight patients that achieved an objective response had responses that lasted 24 or more weeks. Seven percent of the patients that had stable disease as the best response had not have disease progression for at least 24 weeks. When considering all the patients that participate in the trial regardless of the primary tumor, 42 samples were analyzed for PD-L1 status; no objective responses were found in 17 patients with PD-L1 negative tumors, while objective responses were seen in 36% of patients with PD-L1 positive tumors [42].

A second publication of the same phase I trial focused only on the NSCLC cohort with updated results in overall survival, durability of response, and long-term safety published in 2015. The total number of NSCLC patients enrolled was 129. Patients received one of the three doses described above every 2 weeks, in 8-week cycles, for up to 96 weeks. The median of age was 65 years, 42% had a squamous and 57% had a non-squamous histology, 98% had an ECOG performance status of 0–1, and 54% of all the patients had received at least three lines of prior treatment before the first dose of nivolumab. The median overall survival was 9.9 months and the progression-free survival was 2.3 months for all the patients. For all patients included, 1-year survival was 42%, 2-year survival 24%, and 3-year survival 18%, respectively. The chosen doses for further development was nivolumab 3 mg/kg every 2 weeks and the 1-, 2-, and 3-year survival reported for this dose was 56%, 42%, and 27%, respectively with a median overall survival of 14.9 months. The overall response rate was 17% with no statistical difference between histologic subtypes, with a median duration of response of 17 months and a median progression-free survival of 20.6 months. Among all patients, 71% presented an adverse event of any grade (most frequent: fatigue 24%, decreased appetite 12%, and diarrhea 10%) but only 14% had a grade 3 or 4 toxicity (most frequent: fatigue 3%). Defined as adverse event that needed a more frequent monitoring or use of immune suppression treatment or hormonal replace treatment due nivolumab toxicity, 41% of patients presented a "select adverse event" but only 4.7% were grade 3 or 4. Two grade 3–4 and one grade 5 pneumonitis were reported as related with nivolumab. There were three deaths (2%) related with treatment, all of them were associated with pneumonitis [43].

A phase 2 trial, CheckMate 063, was a single arm trial of nivolumab at 3 mg/kg dose given every 2 weeks in squamous NSCLC patients that had received at least two previous lines of treatment for metastatic or unresectable disease. A total of 117 patients participated in this study. The primary endpoint of this study was to evaluate the objective response rate assessed by an independent radiologic review committee. The objective response rate was 14.5% including one patient that achieved a complete response. The reported median time to response was 3.3 months. Median duration of response was not reached. Twenty-six percent of the patients achieved a stable disease as the best radiological response with a median duration of 6 months. The median PFS was 1.9 months, 6-month PFS was 25.9%, and 1-year PFS was 20%. The median OS was 8.2 months with 1-year OS of 40.8%. From patients that provided tumor samples to evaluate PD-1 expression, cutoff points of less or higher than 5%, patients with a higher expression achieved 24% of partial response, 24% of stable disease, and 44% of progressive disease as best response; patients with a

lower PD-1 expression had a 14% of partial response, 20% of stable disease, and 49% of progressive disease as best response to nivolumab treatment. Grade 3–4 adverse events were reported in 17% of patients, the most common were fatigue (4%), diarrhea (3%), pneumonitis (3%), and rash, pruritus, myalgia and anemia (1% each). Twelve percent of treatment-related adverse events led to discontinuation. Two deaths were attributed to nivolumab by investigators, one due to pneumonia and the other to an ischemic stroke; however, both patients had multiple comorbidities and progression of their disease [44]. In a longer follow-up of at least 11 months, median duration of response was still not reached, and no new deaths due to nivolumab were reported [45].

The phase 3 clinical trial CheckMate 017 was a study evaluating stage IIIB or IV squamous NSCLC patients whose disease had progressed through first-line platinum-based doublet chemotherapy. This trial compared nivolumab 3 mg/kg IV every 2 weeks with docetaxel 75 mg/m^2 IV every 3 weeks, with both treatments given until disease progression or unacceptable toxicity. The primary endpoint was OS. Two hundred and sixty patients with an ECOG performance status of 0–1 were randomized. Median age was 62 years in the nivolumab arm and 64 years in the docetaxel arm, and most of the patients included in the study were male. The median overall survival was 9.2 months for nivolumab and 6 months for docetaxel group; 1-year survival for nivolumab and docetaxel were 42% and 24%, respectively. The PFS was 2.8 months for docetaxel and 3.5 months for nivolumab. The objective response rate was 20% for nivolumab and 9% for docetaxel. The median duration of response was 8.4 months for docetaxel and not reached for nivolumab. PD-L1 expression was evaluated using an immunohistochemical assay, Dako North America, from rabbit monoclonal antihuman (Clone 28-8, Epitomics). Any staining at any level was considered as positive. Three levels of positivity for PD-L1 expression were prespecified: 1, 5, and 10%. The authors concluded that PD-L1 expression was neither prognostic nor predictive of benefit for nivolumab. Despite that conclusion, when analyzing the graphics of the original publication it seems to be a trend to benefit in patients treated with nivolumab that had PD-L1 expression greater of 10% when compared with patients with lower levels; the same analysis may be done for patients with PD-L1 expression greater than 5% when compared with patients with lower expression of PD-L1. All grades and grade 3–4 toxicities were much higher for docetaxel arm when compared with nivolumab: 87% versus 59% for all grades, and 56% versus 8% for grade 3–4 adverse events, respectively. Fatigue, decreased appetite and diarrhea were the most common grade 3–4 adverse event reported for nivolumab. Immune-mediated adverse events by organ category were presented in gastrointestinal, pulmonary, and renal in one case each [46].

Due to the benefit in overall survival, the Independent Data Monitoring Committee recommended to stop the trial in January 2015. In March 2015, the FDA approved nivolumab as a second-line treatment for squamous NSCLC patients that have failed first-line platinum-based doublet chemotherapy.

CheckMate 057, with a similar design as CheckMate 017, was a phase 3 clinical trial that compared nivolumab and docetaxel but in non-squamous NSCLC that had progressed during or after platinum-based doublet chemotherapy. Secondary endpoints

included objective response rate, PFS, and efficacy according to PD-L1 expression. Five hundred and eighty-two patients were randomized to receive nivolumab or docetaxel in a 1:1 randomization model. Median overall survival, 12-month overall survival, and 18-month overall survival was 12.2 months, 51%, and 39% for nivolumab-treated patients and 9.4 months, 39%, and 23% for docetaxel, respectively. The response rate was 19 and 12% for nivolumab and docetaxel. Despite median progression-free survival was higher for docetaxel (4.2 vs. 2.3 months), 1-year progression-free survival was 8% for docetaxel and 19% for nivolumab. Grade 3–4 adverse events were much higher for docetaxel (54%) when compared with nivolumab (10%). Fatigue, diarrhea, and nausea were the most common adverse events reported related with nivolumab. In contrast to the squamous NSCLC patients treated in CheckMate 017, PD-L1 expression using the same immunohistochemical assay mentioned before was predictive of outcome for all the endpoints. Subgroup analysis showed also benefit in current or former smokers and in KRAS-mutated patients if being treated with nivolumab, nevertheless, patients that had EGFR mutations, older than 75 years and or never smokers had no clear benefit of the treatment with the monoclonal antibody when compared with docetaxel [47]. Based on the results of this trial, the FDA approved nivolumab for non-squamous NSCLC pretreated patients in October 2015.

An update in 2-year survival for CheckMate 017 and CheckMate 057 was recently presented. Two-year overall survival in CheckMate 017 was 23% for nivolumab versus 8% for docetaxel in squamous NSCLC patients. Two-year overall survival for non-squamous NSCLC patients from CheckMate 057 was 29% for nivolumab and 16% for docetaxel, respectively [48].

In the first-line setting, nivolumab was assessed in the CheckMate 026 trial. This phase 3 trial randomized untreated stage IV or recurrent NSCLC patients in a 1:1 ratio to receive nivolumab at a dose of 3 mg/kg every 2 weeks or a platinum-based chemotherapy every 3 weeks for up to six cycles. Crossover from the chemotherapy arm to the nivolumab arm was permitted. Primary endpoint was the independent central review PFS among patients with a PD-L1 expression of more than 5%. Four hundred and twenty-three patients with a PD-L1 expression level of 5% or more were included. The median progression-free survival was 4.2 months in the nivolumab arm versus 5.9 months with chemotherapy (HR = 1.15; 95% CI, 0.91–1.45; $P = 0.25$), and the median OS was 14.4 months versus 13.2 months (HR = 1.02; 95% CI, 0.80–1.30). A total of 128 of 212 patients (60%) in the chemotherapy group received nivolumab as subsequent therapy. Grade 3–4 treatment-related adverse events occurred in 18% of the patients who received nivolumab and in 51% of those who received chemotherapy. Therefore, nivolumab did not result in a better PFS or OS when compared to chemotherapy in this population [4].

Combination strategies were also investigated in the first-line setting. CheckMate 012 is a phase 1 trial multi-arm that assessed nivolumab as first-line treatment in combination with ipilimumab for NSCLC patients. Patients were randomly assigned (1:1:1) to receive nivolumab 1 mg/kg every 2 weeks plus ipilimumab 1 mg/kg every 6 weeks, nivolumab 3 mg/kg every 2 weeks plus ipilimumab 1 mg/kg every 12 weeks, or nivolumab 3 mg/kg every 2 weeks plus ipilimumab 1 mg/kg every 6 weeks until

disease progression, unacceptable toxicities, or withdrawal of consent [49]. Results of the last two arms were presented where objective responses were achieved in 18 (47% [95% CI 31–64]) patients in the ipilimumab every-12-weeks cohort and 15 (38% [95% CI 23–55]) patients in the ipilimumab every-6-weeks cohort. The median duration of response was not reached in either cohort, with median follow-up times of 12.8 months (IQR 9.3–15.5) in the ipilimumab every-12-weeks cohort and 11.8 months (6.7–15.9) in the ipilimumab every-6-weeks cohort. In patients with PD-L1 of 1% or greater, confirmed objective responses were achieved in 12 (57%) of 21 patients in the ipilimumab every-12-weeks cohort and 13 (57%) of 23 patients in the ipilimumab every-6-weeks cohort. Grade 3–4 treatment-related adverse events occurred in 14 (37%) patients in the ipilimumab every-12-weeks cohort and 13 (33%) patients in the every-6-weeks cohort; the most commonly reported grade 3 or 4 treatment-related adverse events were increased lipase (three [8%] and no patients), pneumonitis (two [5%] and one [3%] patients), adrenal insufficiency (one [3%] and two [5%] patients), and colitis (one [3%] and two [5%] patients) [5].

The CheckMate 227 was an open-label, phase 3 trial, evaluating the combination of nivolumab plus ipilimumab versus chemotherapy among patients with a high tumor mutational burden that was defined as ≥10 mutations per megabase. Patients with previously untreated stage IV or recurrent NSCLC were analyzed for tumor mutational burden using FoundationOne CDx assay. Additionally, patients with a PD-L1 expression of at least 1% were randomly assigned in a 1:1:1 ratio to receive nivolumab plus ipilimumab, nivolumab monotherapy, or chemotherapy. Those with PD-L1 expression of less than 1% were randomly assigned in a 1:1:1 ratio to receive nivolumab plus ipilimumab, nivolumab plus chemotherapy, or chemotherapy. PFS among patients with a high tumor mutational burden was significantly longer with nivolumab plus ipilimumab than with chemotherapy. The 1-year PFS rate was 42.6% with nivolumab plus ipilimumab versus 13.2% with chemotherapy, and the median PFS was 7.2 months (95% CI, 5.5–13.2) versus 5.5 months (95% CI, 4.4–5.8). The HR obtained was 0.58; 97.5% CI, 0.41–0.81; $p < 0.001$ and the objective response rate was 45.3% with nivolumab plus ipilimumab and 26.9% with chemotherapy [6]. The high tumor mutation has become a possible marker to evaluate efficacy of immunotherapy, dissecting the population that will respond better to treatment.

Pembrolizumab

Pembrolizumab (MK-3475, Keytruda®, Merck Sharp & Dohme) is a highly selective IgG4 kappa isotype monoclonal antibody against PD-1. This highly selective antibody binds PD-1 and blocks the PD-1, PD-L1/PD-L2 axis, thus overcoming this major immune checkpoint inhibitor [50]. It was first approved in 2014 for unresectable and metastatic melanoma.

Advanced non-small cell lung cancer patients were assigned to multiple expansion cohorts as part of the phase 1 Keynote 001 clinical trial. Patients with an ECOG performance status of 0–1, adequate organ function, no history of pneumonitis or autoimmune diseases, and no active use of systemic immunosuppressive therapy

were considered to participate in this trial. The primary objectives of this trial were to evaluate the safety, toxicity profile, and activity of pembrolizumab in NSCLC patients. After an amendment, a coprimary endpoint was added to assess the efficacy in patients with NSCLC that expressed high levels of PD-L1. PD-L1 expression was assessed by immunohistochemical 22C3 antibody pharm DX test. Patients were randomized to either pembrolizumab 2 mg/kg every 3 weeks, pembrolizumab 10 mg/kg every 3 weeks, or pembrolizumab 10 mg/kg every 2 weeks, intravenously in a 30 min perfusion.

Of the 495 randomized patients that received at least one dose of pembrolizumab, any-grade adverse events were presented in 70% of the patients, grade 3 or higher adverse events were reported in 9.5% of patients. The most common any-grade adverse events were fatigue, pruritus, and decreased appetite. Most frequent treatment-related adverse events reported were infusion reactions 2%, hypothyroidism 6.9% and pneumonitis 3.6% including 1.8% grade 3 and 1 death for this reason. Regardless of the dose, schedule, and histology, similar response rate were found among the three arms. The overall response rate was 19.4% (18% for previous treated and 24.8% for untreated patients) and overall stable disease was 21.8%. Response rate was also higher in current or former smokers (22.5%) as compared with never smoker patients (10.3%). Median duration of response was 12.5 months (10.4 months for previous treated and 23.3 months for untreated patients). Overall median progression-free survival and median overall survival was 3.7 months (3 months for previous treated and 6 months for untreated patients) and 12 months (9.3 months for previous treated and 16.2 months for previous untreated patients), respectively. Tumor samples assessment showed that PD-L1 expression 1–49% was present in 37, 6% of patients and higher of 50% was present in 23.2% of patients. The objective response rate (45.2%) was higher in patients that overexpressed PD-L1 (50% or higher) when compared with patients that had PD-L1 expression of 1–49% or less than 1%. Median progression-free survival for the group with high PD-L1 expression was 6.3 months and median overall survival was not reached [51].

Recent update from Keynote 001 regarding overall survival in patients with PD-L1 expression of 1–49% showed a median overall survival of 11.3 months in previous treated and 22.1 months in untreated patients. Median overall survival for PD-L1 expression of 50% or higher was 15.4 months for previous treated and still not reached for untreated patients [52].

Based on these results, in October 2015, FDA approved pembrolizumab for metastatic NSCLC patients that failed to a first line of cytotoxic chemotherapy and presented with a positive PD-L1 expression.

Conducted in 24 countries, Keynote 010 was an open-label phase 2–3 trial that compared, in NSCLC patients that had failed to at least one prior line of platinum-based doublet chemotherapy, pembrolizumab with docetaxel. All patients had to have at least 1% of PD-L1 expression in their tumors evaluated by immunohistochemical assay (22C3 antibody pharm DX test) and measurable disease according to RECIST 1.1. Patients were randomized to receive pembrolizumab 2 mg/kg every 3 weeks, pembrolizumab 10 mg/kg every 3 weeks, or docetaxel 75 mg/m^2 every 3 weeks. Primary endpoints were overall survival and progression-free survival in

the total population and in the group of patients that have a high expression of PD-L1 (50% or higher). Nine hundred and ninety-one NSCLC patients (22% squamous) received at least one dose of pembrolizumab or docetaxel. Twenty-eight percent of patients had a PD-L1 expression of at least 50%. In the total population group, overall survival was higher in both groups of pembrolizumab treated patients when compared with docetaxel with a HR 0.71 for pembrolizumab 2 mg/kg dose ($p = 0.0008$) and a HR 0.61 for pembrolizumab 10 mg/kg dose ($p = 0.0001$). Median overall survival and 1-year survival was 10.4 months and 43.2%, 12.7 months and 52.3%, 8.5 months and 34.6% for pembrolizumab 2 mg, pembrolizumab 10 mg, and docetaxel arms, respectively. No differences in overall survival were between both arms containing pembrolizumab. In subgroups analysis, there was a clear benefit for the adenocarcinoma patients; however, there was not a clear benefit in overall survival for squamous NSCLC patients.

Benefit in overall survival was higher in patients treated with pembrolizumab with high expression of PD-L1 (at least 50%). When compared with docetaxel the HR of pembrolizumab 2 mg was 0.54 ($p = 0.0002$) and HR 0.5 ($p = 0.0001$) for 10 mg/kg dose. Median overall survival in patients with high expression of PD-L1 was for pembrolizumab 2 mg/kg, for pembrolizumab 10 mg, and for docetaxel 14.9 months, 17.3 months, and 8.2 months, respectively. Progression-free survival was not statistically superior for the pembrolizumab arms when compared with docetaxel in the total population; however, it was significantly higher in patients with high expression of PD-L1 (HR 0.59) for both groups of pembrolizumab. Median progression-free survival was 5 months for pembrolizumab 2 mg/kg, 5.2 months for pembrolizumab 5.3 mg/kg, and 4.1 months for docetaxel. Objective response rate was significantly higher either for both pembrolizumab arms than for docetaxel. That was seen in the total study population and in patients with PD L1 expression of 50% or higher as well. For pembrolizumab 2 mg, pembrolizumab 10 mg, and docetaxel, response rates for the total population and for higher PD-L1 population were 18 and 30%, 18 and 29%, 9 and 8%, respectively. There were no complete responses in none of the three treated groups. Toxicity was significantly lower in both pembrolizumab arms when compared with docetaxel. Grade 3–5 adverse events and toxicity that led to treatment discontinuation was reported as follows: 13 and 4% for pembrolizumab 2 mg, 16 and 5% for pembrolizumab 10 mg, 35 and 10% for docetaxel arm. Immune-related toxicity was similar for pembrolizumab 2 mg (20%) and for pembrolizumab 10 mg (19%). Most common immune-related adverse events reported were hypothyroidism, hyperthyroidism, and pneumonitis. Grade 3–5 adverse events reported in more than 1% in both pembrolizumab arms were pneumonitis and skin reactions. Two treatment-related deaths were reported for pembrolizumab 2 mg (one pneumonitis and one pneumonia) and three deaths for pembrolizumab 10 mg (one myocardial infarction, one pneumonia, and one pneumonitis) [53].

Recent updated reports of Keynote 010 showed a statistically greater outcome in overall survival, progression-free survival, and response rate for patients that present PD-L1 expression of 75% or higher when compared with subgroups with lower expression (PD-L1 expression 50–74%, 25–49%, and 1–24%). No differences in these outcomes were reported for docetaxel-treated group regardless of the level of PD-L1 expression [54].

Benefit in overall survival in pembrolizumab-treated patients was not driven solely by the PD-L1 expression of 50% or higher. A recent report confirmed that patients from Keynote 010, that were treated with pembrolizumab, had benefit in overall survival when compared with docetaxel (HR 0.79 with 9.4 months in median overall survival for pembrolizumab 2 mg/kg dose, HR 0.71 with median overall survival of 10.8 months for pembrolizumab 10 mg/kg dose, versus median overall survival of 8.6 months for docetaxel arm) [55].

About the importance to provide a new tissue sample or not, to evaluate the PD-L1 expression versus using archived samples to assess this expression by immunohistochemistry, no differences in overall survival were seen between patients with archived or new samples and not significantly difference in PD-L1 expression of 50% or higher was found regardless if the biopsy provided was archived or from a fresh tissue sample [56].

Keynote 024 is a phase 3 trial that included 305 patients not previously treated for an advanced NSCLC with PD-L1 expression on at least 50% of tumor cells and no sensitizing mutation of the EGFR gene or ALK translocation to receive either pembrolizumab (at a fixed dose of 200 mg every 3 weeks) or the investigator's choice of platinum-based chemotherapy. Crossover from the chemotherapy group to the pembrolizumab group was permitted. The primary endpoint, PFS, was assessed by means of blinded, independent, central radiologic review. Secondary endpoints were overall survival, objective response rate, and safety. Median PFS was 10.3 months (95% CI, 6.7 to not reached) in the pembrolizumab group versus 6.0 months (95% CI, 4.2–6.2) in the chemotherapy group with a HR = 0.50; 95% CI, 0.37–0.68; $p < 0.001$. The estimated rate of overall survival at 6 months was 80.2% in the pembrolizumab group versus 72.4% in the chemotherapy group (HR = 0.60; 95% CI, 0.41–0.89; $p = 0.005$). The response rate was higher in the pembrolizumab group than in the chemotherapy group (44.8% vs. 27.8%), and the median duration of response was longer (not reached [range, 1.9+ to 14.5+ months] vs. 6.3 months [range, 2.1+ to 12.6+]). Regarding toxicity, treatment-related adverse events of any grade were less frequent in the pembrolizumab arm, occurring in 73.4% versus 90.0% of patients, were grade 3, 4, or 5 treatment-related adverse events, and were present in 26.6% versus 53.3% [7]. An updated analysis after 25 months of follow-up was later presented showing a OS with pembrolizumab of 30.2 months versus 14.2 months with chemotherapy, representing a 37% reduction in the risk of death (hazard ratio, 0.63; 95% CI, 0.47–0.86; $p = 0.002$). The 24-month OS rate was 51.5% versus 34.5% favoring the pembrolizumab arm. At 12 months, the OS rate was 70.3% in the pembrolizumab arm compared with 54.8% in the chemotherapy group. The ORR was 45.5% (95% CI, 37.4–53.7) with pembrolizumab compared with 29.8% (95% CI, 22.6–37.8) in the chemotherapy group. Median duration of response was not reached in the pembrolizumab group (range, 1.8+ to 20.6+ months) compared with 7.1 months (range, 2.1+ to 18.1+ months) in the chemotherapy group [8].

Keynote 042 is a phase 3 clinical trial for the first-line metastatic or unresectable NSCLC (squamous and non-squamous histology), in patients that are not amenable for curative treatment and had a PD-L1 expression of at least 1%. Patients were assigned to receive pembrolizumab as a monotherapy versus chemotherapy

(carboplatin plus paclitaxel or carboplatin plus pemetrexed). PD-L1 levels were assessed by tumor proportion score (TPS). The primary endpoint was OS with TPS of ≥50%, ≥20%, and ≥1%. The study has met its endpoint and the result will be presented in a near future [57, 58].

Combination trials have also been evaluated with pembrolizumab. Keynote 189 is a double-blind, phase 3 trial, that assigned 616 metastatic non-squamous NSCLC patients without sensitizing EGFR or ALK mutations who had received no previous treatment for metastatic disease in a 2:1 ratio to receive pemetrexed and a platinum-based drug in combination with either 200 mg of pembrolizumab or placebo every 3 weeks for four cycles, followed by pembrolizumab or placebo for up to a total of 35 cycles plus pemetrexed maintenance therapy. Crossover to pembrolizumab monotherapy was permitted among the patients in the placebo-combination group who had verified disease progression. The primary endpoints were overall survival and progression-free survival, as assessed by blinded, independent central radiologic review. Overall survival at 12 months was 69.2% (95% CI, 64.1–73.8) in the pembrolizumab-combination group versus 49.4% (95% CI, 42.1–56.2) in the placebo-combination group (HR = 0.49; 95% CI, 0.38–0.64; $p < 0.001$) after a median follow-up of 10.5 months. The benefit of the pembrolizumab combination was observed in all subgroups that were analyzed, including those with a PD-L1 tumor proportion score of less than 1% (12-month OS rate, 61.7% vs. 52.2%; HR = 0.59; 95% CI, 0.38–0.92), a score of 1–49% (12-month OS rate, 71.5% vs. 50.9%; HR = 0.55; 95% CI, 0.34–0.90), and a score of 50% or greater (12-month OS rate, 73.0% vs. 48.1%; HR = 0.42; 95% CI, 0.26–0.68). Median PFS was 8.8 months (95% CI, 7.6–9.2) in the pembrolizumab-combination group and 4.9 months (95% CI, 4.7–5.5) in the placebo-combination group (HR = 0.52; 95% CI, 0.43–0.64; $p < 0.001$). Adverse events of grade 3 or higher occurred in 67.2% of the patients in the pembrolizumab-combination group and in 65.8% of those in the placebo-combination group. The frequency of deaths attributed to pneumonitis in this trial was consistent with the frequency previously observed with pembrolizumab monotherapy in advanced NSCLC [9].

Anti-PD-L1 Inhibitors

An interesting strategy, similar to PD-1 blockade, is the chance to block PD-L1 using monoclonal antibodies that bind this ligand. The PD-L1 antibodies do not prevent PD-1 from interacting with PD-L2 and CD80, which seems to play a role in controlling inflammation and protect normal lung tissue from excessive damage when immune system is activated [59].

This different mechanism of action of the anti-PD-L1 inhibitors, when compared with PD-1 inhibitors, can lead to a more reduced immune-related toxicity and also, by blocking the interaction between PD-L1 and CD80, can help to suppress another negative control on T cells that can theoretically maximize the monoclonal antibody's activity [60]. This has not been proven clinically.

There have been several drugs under research.

Durvalumab (MEDI4736)

Durvalumab is a high affinity human IgG1 that selectively blocks PD-L1 binding to PD-1 and CD80 without binding to PD-L2, decreasing the risk of immune-related toxicity due to PD-L2 inhibition.

In a phase 1 dose escalation, cohort expansion, clinical trial, safety and efficacy of durvalumab was assessed in NSCLC pretreated and treatment-naïve patients. Forty-three percent of patients presented grade 1–2 adverse events; however, no grade 3–5 pneumonitis was reported and no differences in toxicity between pretreated or treatment-naïve patients were seen. Preliminary results of 13 first patients that underwent treatment in the different cohorts showed 3 partial responses and 2 other patients that achieved tumor shrinkage without resulting in partial response using immune RECIST criteria. Expansion cohort was opened to recruit at least 300 patients [61].

Recently an update from the phase 1–2 clinical trial was reported in which 198 NSCLC patients (116 non-squamous and 82 squamous histology) were treated using durvalumab in a dose of 10 mg/kg intravenously every 2 weeks, until disease progression, unacceptable toxicity or after 1-year of treatment, whatever first, with the chance to retreat patients if they failed after 12 months of treatment. The objective response rate was 14% but it was higher in the PD-L1 positive patients (23%). By histology, response rate was higher in squamous than in non-squamous histology (21% and 10%, respectively). Duration of response range was from 0.1 to 35 weeks. Any grade toxicity was reported in 48% of patients, most common reported adverse events were fatigue (14%), decreased appetite (9%), and nauseas (8%). Six percent of patients had a grade 3–4 toxicity and only 2% of patients were discontinued treatment due to toxicity. From the total of patients treated, there was only two pneumonitis reported [62].

A recent report based on a treatment-naïve population showed an objective response rate of 25% (26% in squamous and 25% in non-squamous NSCLC) and a disease control rate of 12 or more weeks of 56%. Grade 3 or higher toxicity was reported in 9% of patients with 7% of treatment discontinuation due to toxicity with two cases of diarrhea that led to stop treatment [63].

As monotherapy, durvalumab has shown the most promising results in locally advanced stage III patients after receiving chemoradiotherapy. This phase 3 study randomly assigned patients in a 2:1 ratio to receive durvalumab at a dose of 10 mg per kilogram or placebo every 2 weeks for up to a year. These treatments were given between 1 and 42 days after a definitive treatment of chemoradiotherapy. Two primary endpoints were explored: PFS and OS. The study included 709 patients that received treatment, 473 receiving durvalumab, and 236 receiving placebo. The results were published in which median PFS was 16.8 months (95% CI, 13.0–18.1) with durvalumab versus 5.6 months (95% CI, 4.6–7.8) with placebo (HR = 0.52; 95% CI, 0.42–0.65; $p < 0.001$); the 12-month PFS rate was 55.9% versus 35.3%, and the 18-month PFS rate was 44.2% versus 27.0%. The median duration of response was longer for the durvalumab arm (72.8% vs. 46.8% of the patients had an ongoing response at 18 months). The median time to distant metastasis or death was longer with durvalumab 23.2 months than with placebo (14.6 months;

$p < 0.001$). Adverse events were also important to evaluate given the nature of the study, in which a treatment was given after a definitive management where there was no prior recommendation of treatment continuation. Grade 3 or 4 adverse events occurred in 29.9% of the patients who received durvalumab and 26.1% of those who received placebo; the most common adverse event of grade 3 or 4 was pneumonia (4.4% and 3.8%, respectively). Also, a total of 15.4% of patients in the durvalumab group and 9.8% of those in the placebo group discontinued the study drug because of adverse events [10]. The positivity of this trial has been possibly the most important advance in locally advanced disease in the last decade.

Combining an anti-PD-L1 with an anti-CTLA-4 antibody is a promising alternative in NSCLC patients that is under evaluation. A multicenter non-randomized, open-label phase 1b study assessed the safety and antitumor activity of durvalumab plus tremelimumab in 102 locally advanced or metastatic NSCLC patients. Durvalumab was given in doses of 3 mg/kg, 10 mg/kg, 15 mg/kg or 20 mg/kg every 4 weeks or in a dose of 10 mg/kg every 2 weeks; tremelimumab was given in doses of 1, 3, or 10 mg/kg every 4 weeks for six doses, then after every 12 weeks for three doses. The maximum tolerated dose was exceeded in the cohort that received durvalumab 20 mg/kg every 4 weeks plus tremelimumab 3 mg/kg every 4 weeks with two of six patients with dose-limiting toxicity (one patient with grade 3 elevated transaminases and one patient with grade 4 increased lipase). Toxicity led to discontinuation of treatment in 26% of the patients. The most common any-grade adverse events reported were diarrhea (32%), fatigue (24%), and pruritus (21%). Most common grade 3 or grated reported toxicities were diarrhea (11%), colitis (9%), and increased lipase (8%). Three of 22 deaths during the study period were reported as attributed to treatment. Based on safety data, the dose chosen for the expansion phase dose was durvalumab 20 mg/kg plus tremelimumab 1 mg/kg. Of the 63 patients that were assessed for tumor response, 17% achieved an objective response (including 5% in PD-L1 negative patients) and disease control rate was achieved in 29% of patients. Based on this the authors of this trial concluded that PD-L1 status might not predict the response to durvalumab plus tremelimumab combination [64].

Licensed by Astra Zeneca, durvalumab is currently under study in different clinical trials for NSCLC patients, including the TATTON trial where durvalumab is evaluated with osimertinib, either as monotherapy or in combination with tremelimumab.

Atezolizumab (MPDL3280A)

Another anti-PD-L1 agent is atezolizumab, a human IgG1 monoclonal antibody that contains a mutated Fc domain designed to avoid Fc-receptor binding and therefore any PD-L1-targeted ADCC [65].

In a phase I expansion study, squamous and non-squamous pretreated NSCLC patients were treated with atezolizumab at doses between 1 and 20 mg/kg. Reported grade 3–4 adverse events included pericardial effusion (6%), dehydration (4%), dyspnea (4%), and fatigue (4%). No treatment-related deaths occurred. The reported objective response rate by RECIST 1.1 was 24%. Twenty-four-week progression-

free survival was 48%. Four over four patients that had PD-L1 positive status achieved objective response (100%), nevertheless PD-L1 negative patients (4/26) achieved an overall response rate of 15% with progression disease of 58% [66].

The expanded trial which included 85 NSCLC patients with both squamous and non-squamous histology, within a study that included other cancer types such as melanoma and renal cell carcinoma, was performed. NSCLC patients were treated with atezolizumab every 3 weeks, achieving an objective response rate of 21%. Current and former smoker had a higher response rate than never smokers (42% vs. 10%, respectively). Patients with higher expressions of PD-L1 levels achieved better responses compared to whom did not. For all the patients treated in this trial, including NSCLC and other tumor types, any grade toxicities were reported in 70% of the patients. The most common adverse events reported were fatigue (24%), decreased appetite (11%), nauseas (11%), pyrexia (11%), diarrhea (10%), and rash (10%); grade 3–4 toxicities were reported in 39% of patients and included dyspnea (4%), anemia (3.6%), fatigue (3.2%), and hyperglycemia (2.5%) [67].

Clinical outcomes in distinct cancer types with high levels of PD-L2 expression have also showed a superior benefit with atezolizumab treatment [68].

The combination of atezolizumab plus chemotherapy in the first line of treatment in NSCLC patients has been tested in a phase 1b trial. Patients received atezolizumab 15 mg/kg intravenously every 3 weeks plus 4–6 doses of platinum-based chemotherapy followed of atezolizumab as maintenance therapy. Up to 13% of patients presented grade 3–4 toxicity, most of them hematological and related with chemotherapy. One death due to candidemia after a prolonged neutropenia was reported. Overall response rate was different into groups of chemotherapy treatment but it ranged between 60 and 75%, responses were considered as not related to PD-L1 status [69].

The phase 2 clinical trial BIRCH was an open-label multicenter study that assessed the safety and efficacy of atezolizumab in NSCLC patients that express PD-L1. This trial included 667 treatment-naïve and pretreated patients. PD-L1 status was assessed by an immunohistochemical assay developed by Roche Diagnostics that measures tumor cells (TCs) and tumor-infiltrating immune cells (ICs), therefore its results are interpreted by a score that included both components and were reported as TC 0 (TC0 < 1%), 1 (TC1 ≥ 1% and <5%), 2 (TC2 ≥ 5% and <50%) or 3 (TC3 ≥ 50%) and IC 0 (IC0 < 1%), 1 (IC1 ≥ 1% and <5%), 2 (IC2 ≥ 5% and <10%) or 3 (IC3 ≥ 10%). Eligible patients for this trial were patients with a TC 2/3 or IC 2/3. Patients included received atezolizumab at 1200 mg intravenously every 3 weeks. The primary endpoint was objective response rate. Patients that scored TC 3/IC 3 had higher responses rates than patients that presented TC 2/3 or IC 2/3 in the first line (26% vs. 19%), second line (24% vs. 17%), and third line or further of treatment (27% vs. 17%) [70].

The POPLAR trial was a phase 2 study that compared atezolizumab versus docetaxel in locally advanced or metastatic NSCLC that had progressed after a first line of treatment, regardless of the PD-L1 status assessed by the same immunohistochemical assay that was mentioned above. Two hundred and eighty-seven patients were enrolled in the trial receiving atezolizumab at a fixed dose of 1200 mg every

3 weeks. POPLAR's primary endpoint was overall survival. Atezolizumab achieved higher survival than docetaxel in all the subgroups of patients that were PD-L1 positive: median overall survival for any expression 15.5 months versus 9.2 months (HR 0.59 $p = 0.005$), medium (TC2/3 or IC2/3) and high (TC3 or IC3) expression 15.1 months versus 7.4 months (HR 0.54 $p = 0.014$), high expression 15.5 months versus 11.1 months (HR 0.49 $p = 0.068$). For PD-L1 negative patients (TC 0 and IC 0), there was no difference in median overall survival for atezolizumab and docetaxel (9.7 months for both groups) [71].

A recent update of POPLAR trial showed an increase in the separation of curves with improved overall survival in favor of atezolizumab when compared with docetaxel (ITT population median overall survival 12.6 months versus 9.7 months ($p = 0.011$); TC3 or IC3 median overall survival not reached versus 11.1 months ($p = 0.033$). Regarding histology, there was no significant difference between histologies, with both histologic subtypes (squamous vs. non-squamous) favoring atezolizumab over docetaxel in overall survival [72].

The OAK trial was a phase 3, open-label, second or higher line international trial. Patients included had a stage IIIB or IV squamous or non-squamous NSCLC who had received one or two previous chemotherapy regimens and no previous anti-CTLA-4, anti-PD-L1, or anti-PD-L1 therapy. Patients were randomly assigned in a 1:1 to either atezolizumab 1200 mg or docetaxel 75 mg/m^2 every 3 weeks. Coprimary endpoints were OS in the intention-to-treat (ITT) and PD-L1-expression population TC1/2/3 or IC1/2/3 (≥1% PD-L1 on tumor cells or tumor-infiltrating immune cells). One thousand two hundred and twenty-five patients were recruited where 425 patients were randomly assigned to receive atezolizumab and 425 patients were assigned to receive docetaxel. OS was significantly longer in patients who had received atezolizumab in both the ITT and PD-L1-expression populations. In the ITT population, OS was improved with atezolizumab compared with docetaxel where the median OS was 13.8 months (95% CI 11.8–15.7) versus 9.6 months (8.6–11.2); HR = 0.73 (95% CI 0.62–0.87); $p = 0.0003$. OS in the TC1/2/3 or IC1/2/3 population was improved with atezolizumab ($n = 241$) compared to docetaxel ($n = 222$); median OS was 15.7 months (95% CI 12.6–18.0) with atezolizumab versus 10.3 months (8.8–12.0) with docetaxel; HR = 0.74 (95% CI 0.58–0.93); $p = 0.0102$. Patients in the PD-L1 with TC0 and IC0 also had a positive result with improved survival favoring atezolizumab with a median OS of 12.6 months versus 8.9 months; HR = 0.75 (95% CI 0.59–0.96). OS improvement difference was similar in the squamous and non-squamous populations. Regarding side effects, fewer patients had treatment-related grade 3 or 4 adverse events with atezolizumab (15% of patients) versus docetaxel (43% of patients). One treatment-related death from a respiratory tract infection was reported in the docetaxel group [11].

Atezolizumab has also been recently evaluated in combination with bevacizumab and chemotherapy among patients with previously untreated metastatic non-squamous NSCLC regardless of PD-L1 expression. The IMpower 150 trial is an international, open-label, phase III study which randomized 1202 patients in a 1:1:1 ratio into three treatment arms: atezolizumab plus carboplatin plus paclitaxel (ACP), atezolizumab plus bevacizumab plus carboplatin plus paclitaxel (ABCP), or bevaci-

zumab plus carboplatin plus paclitaxel (BCP), each administered for 4–6 cycles. After induction chemotherapy, patients continued to receive atezolizumab, bevacizumab, or both until disease progression or intolerable toxicity. Primary endpoints were progression-free survival in the intention-to-treat population with wild type (WT) genotype (no EGFR or ALK genomic alterations) and among patients in the WT population with high expression of an effector T cell gene signature (Teff-high WT population), as well as overall survival in the WT population. The Teff gene signature was defined as the expression of PD-L1, CXCL9 and IFN-γ messenger RNA. In the WT population, median progression-free survival was significantly longer in the ABCP arm than in the BCP arm (8.3 vs. 6.8 months, HR 0.62, 95% CI 0.52–0.74, $p < 0.001$). In the Teff-high WT population, median progression-free survival was significantly longer in the ABCP group compared to the BCP group (11.3 vs. 6.8 months, HR 0.51, 95% CI 0.38–0.68, $p < 0.001$). In subgroup analysis, prolonged progression-free survival was also noted irrespective of PD-L1 status, including those with no PD-L1 expression, low PD-L1 expression, and low Teff gene signature expression. Notably, in an analysis of patients with EGFR mutations or ALK translocations ($n = 108$), median progression-free survival was also longer in the ABCP arm compared to the BCP arm (9.7 vs. 6.1 months, HR 0.59, 95% CI 0.37–0.94). Among the wild type population, OS was found to be significantly longer in the ABCP arm compared to the BCP arm (19.2 months vs. 14.7 months, HR 0.78, 95% CI 0.64–0.96, $p = 0.02$). Grade 3 or 4 treatment-related adverse events occurred in 55.7% of patients in the ABCP arm and 47.7% of the BCP group. The safety profile of the ABCP arm was felt to be consistent with the known safety risks of each of the individual drugs [73]. The data from this study suggest that the addition of cytotoxic chemotherapy to immune checkpoint inhibitors may enhance the effects of PD-1/PD-L1 inhibition.

Avelumab

Avelumab (MSB0010718C) is a fully human anti-PD-L1 IgG1 monoclonal antibody and has a native Fc receptor for ADCC [74].

A phase I, open-label, parallel-group expansion study of avelumab was conducted to assess the tolerability and safety of avelumab in metastatic or local advanced solid tumors that included NSCLC patients but also gastric, ovarian, melanoma, and breast cancer patients. Avelumab was given a 10 mg/kg dose every 2 weeks. Four hundred and eighty patients were treated in this trial and 68% of them present an adverse event any grade, most frequent toxicities reported were fatigue (20%), nausea (13%), infusion-related reaction (9%), diarrhea (7%), chills (7%), decreased appetite (6%), pyrexia (5%), influenza-like illness (5%), and arthralgia (5%). Thirty-four patients were discontinued of treatment due to adverse events including eight patients that presented infusion reactions. Drug-related toxicity grade 3 or higher was reported in 12% of patients and the most common toxicities reported were anemia (5), fatigue (5), increased GGT (4), infusion reactions (4), increased lipase (4), and decreased lymphocytes (3). Immune-related toxicities

were reported in 11.7% of patients and the most common were hypothyroidism (4.0%) and pneumonitis (1.5%) [75].

Inside this study, stage III B or IV NSCLC patients previously treated with a platinum-based doublet were considered to receive avelumab 10 mg/kg every 2 weeks until complete response, disease progression, or unacceptable toxicity. One hundred and eighty-four NSCLC patients were included (62% adenocarcinoma, 29% squamous carcinoma). Seventy-five percent of patients presented at least one any-grade adverse event. Most common toxicities reported were fatigue, nausea, infusion-related reactions, chills, decreased appetite, and diarrhea. Drug-related toxicity grade 3–4 was present in 12% of patients including four cases of infusion reactions. Three drug-related deaths were reported (radiation pneumonitis, acute respiratory failure, and disease progression). Response rate and stable disease were observed in 12 and 38% of patients (14.4% of response rate in PD-L1 positive and 10% in PD-L1 negative patients). Overall progression-free survival was 11.6 weeks (11.7 weeks in PD-L1 positive and 5.9 weeks in PD-L1 negative patients) [76].

In a phase 1b trial, avelumab was tested as first line of treatment in 145 local advanced or metastatic NSCLC patients (63% adenocarcinoma, 27 squamous) without EGFR or ALK mutations, regardless of the PD-L1 status.

Patients received avelumab 10 mg/kg intravenously every 2 weeks until progression or unacceptable toxicity. All grade toxicities were reported in 56% of patients. Most common adverse events were infusion reactions (16%) and fatigue (14%). Grade 3–4 toxicities were reported in 9% of the patients. No deaths related to treatment were observed. Overall response rate assessed by RECIST 1.1 was reported in 18.7% of patients (1 complete response and 13 partial responses), stable disease was reported in 45% of patients. All reported responses were achieved in PD-L1 positive patients without any response in PD-L1 negative patients. Median progression-free survival was 11.6 weeks for all the treated population [77].

Currently, a phase 3 clinical trial comparing avelumab with docetaxel as second line of treatment for PD-L1 positive NSCLC patients is ongoing [78].

BMS-936559

BMS-936559 is a fully human IgG4 antibody that inhibits binding of PD-L1 to PD-1 and CD80, binding PD-L1 but also CTLA-4 and CD28 with high affinity [59].

This drug was tested in a phase 1 dose escalation and cohort expansion trial including melanoma, NSCLC, renal cell carcinoma patients and others (ovarian, pancreatic, colorectal cancer). There was 8.6% of grade 3–4 toxicity without deaths due to treatment. Some adverse events of special interest reported were hypothyroidism, hepatitis, sarcoidosis, endophthalmitis, and myasthenia gravis. Objective responses were observed in heavily pretreated patients including responses lasting longer than 1 year [79]. Despite this drug is not currently being studied in cancer patients, there are clinical trials ongoing for sepsis treatment.

Immunotherapy and NSCLC: Milestones, Concerns, Fears, and Challenges

Non-small cell lung cancer is unfortunately the most common malignancy worldwide. Official records by Globocan showed that in 2012 there was an incidence, including both sexes, of 1,824,701 new cases around the world and 1,589,925 deaths in the same year for this disease. In other words, for every 100 persons that have been diagnosed with lung cancer there will be 87 persons that will die due to lung cancer in a 12 month time period. For both sexes together and in men, non-small cell lung cancer is the leading cause of mortality by cancer and the second cause of mortality by cancer in women [80]. In the United States, there is a trend to decrease in incidence and mortality due to NSCLC since 2012. Anti-tobacco laws and regulations are playing probably a major role in this trend to "improve" of the curves; however, there was reported in the United States an 5-year survival for lung cancer of only 17.7% for the period 2006–2012, with 224,390 new cases estimated for 2016 and 158,080 deaths in the same year representing 26.5% of mortality for cancer in this country [81].

Since 1980s and until the first half of the 2000s decade, very few steps that had a real impact in the prognosis of unresectable or metastatic NSCLC patients were given: some new chemotherapy regimens (always in first-line platinum-based doublets); attempts to add antiangiogenics to chemotherapy regimens; development of second-line cytotoxic chemotherapies. However, those steps did not achieve a great impact in overall survival and obviously lesser impact in 5-year survival rates. By the second half of the 2000s targeted therapies, in the beginning directed against EGFR mutations and years later against ALK translocations, have taken a place in the treatment of this malignancy, achieving a high impact in overall survival in this population of patients, that represents approximately one-fourth to one-fifth of the entire population of non-small cell lung cancer worldwide, with disparities by regions probably due to genetics and tobacco consumption.

We have been witnesses of the most revolutionary milestone of the systemic cancer treatment: the emergence of immunotherapy. Unexpected first results in melanoma patients were published in 2010, changing the paradigm of how to treat this malignancy. Pooled analysis showed that one-fourth of the patients that had been treated with ipilimumab are alive for more than 3 years, with a clear plateau in the survival curve. It is too early yet to talk about "the cure of cancer," nonetheless it seems that immunotherapy in general is given an approach to this scenario. We are currently under a storm of information that many times exceeds the capability of analysis and comprehension. New drugs are emerging and clinical trials that are looking for testing them are under development.

First reports and approval in NSCLC of immunotherapy drugs are relatively new, time will be needed to assess a longer term benefit; however, with the current information we already can say that there must be a change in the paradigm of how to treat NSCLC patients that are not amenable for curative options.

Lung cancer cells have multiple immunosuppressive mechanisms that are critical to escape of the immune system and survive. Anti-CTLA-4 such as ipilimumab, drug that changed the paradigm in melanoma treatment, when tested in clinical trials did not show the expected benefit in non-small cell lung cancer patients. Nevertheless, other checkpoint inhibitors such as anti-PD-1 and anti-PD-L1 are emerging. These drugs do not attack directly the tumor cell as cytotoxic chemotherapy does, they work by suppression of the main mechanisms involved in immune-tolerance and tumor evasion from immune response.

In NSCLC anti-PD-1 and anti-PD-L1 monoclonal antibodies have shown significant activity, significant outcomes in survival, long lasting responses, and good safety profile when compared with cytotoxic chemotherapy, including naïve and pretreated patients with squamous and non-squamous histology (Tables 3.1 and 3.2). Moreover patients not expressing PD-L1 in their tumors, when treated with anti-PD-1 drugs, achieve similar responses to patients treated with chemotherapy, but patients with high levels of PD-L1 expression have much better results when compared with standard treatment.

Identification of predictive biomarkers to select patients most likely responding to immunotherapies is currently being investigated. Because of the critical role of PD-1/PD-L1 pathway activation in downregulating T cell activity, several investigations have focused on tumor microenvironment components [23–88]. PD-L1 is upregulated in selected solid tumors, including squamous and non-squamous non-small cell lung cancers, and it can be detected by immunohistochemistry on tumor cells (TCs) and immune cells (ICs).

Both anti-PD-1 pembrolizumab and anti-PD-L1 atezolizumab show a greater impact in outcomes in PD-L1 positive patients. Nivolumab, however, got approval without needing PD-L1 positive demonstration, even though there is a trend of benefit in PD-L1 positive patients, mainly in adenocarcinoma histology. One big problem is how to translate the results of the different trials in order to define what

Table 3.1 Pivotal second-line phase III immunotherapy trials in advanced NSCLC

Trial	Histology, PD-L1 expression requirement	Drugs	Number of patients	Median PFS (months)	Median OS (months)
CheckMate 017	Squamous	Nivolumab	135	3.5	9.2
		Docetaxel	137	2.8	6.0
CheckMate 057	Non-squamous	Nivolumab	292	2.3	12.2
		Docetaxel	290	4.2	9.4
KEYNOTE-010	NSCLC, ≥1%	Pembrolizumab 2 mg/kg	344	3.9	10.4
		Pembrolizumab 10 mg/kg	346	4.0	12.7
		Docetaxel	343	4.0	8.5
OAK	NSCLC	Atezolizumab	425	2.8	13.8
		Docetaxel	425	4.0	9.6

NSCLC non-small cell lung cancer, *PFS* progression-free survival, *OS* overall survival

Table 3.2 Pivotal first-line phase III immunotherapy trials in advanced NSCLC

Trial	Histology, PD-L1 expression requirement	Drug	Number of patients	Median PFS (months)	Median OS (months)
KEYNOTE-024	NSCLC, ≥50%	Pembrolizumab	154	10.3	30.2
		Platinum-based chemotherapy	151	6.0	14.2
KEYNOTE-189	Non-squamous	Pembrolizumab + platinum + pemetrexed	410	8.8	NR
		Placebo + platinum + pemetrexed	206	4.9	11.3
IMpower 150	Non-squamous	Atezolizumab + carboplatin + paclitaxel	348	N/A	N/A
		Atezolizumab + bevacizumab + carboplatin + paclitaxel	356	8.3	19.2
		Bevacizumab + carboplatin + paclitaxel	336	6.8	14.7

NSCLC non-small cell lung cancer, *PFS* progression-free survival, *OS* overall survival, *NR* not reached, *N/A* not available

should be considered as PD-L1 positive, which ought to be the cutoff point and then how to define the best treatment for every patient [89]. This is a confusing situation. We cannot affirm if an anti-PD-1 is more effective than the other just for the published results of the different trials. All the anti PD-1s approved and the anti-PD-1s and anti-PD-L1s under research and development use different assays to measure the levels of PD-L1 expression [90]. Probably in a short time, some of the immunotherapy drugs under development will be approved and the decision of treatment will become harder. PD-L1 seems to be a predictive biomarker; however, when there are several immunohistochemical assays for just one biomarker, it is difficult to decide which one to use, and it is also important to understand that currently every assay is linked to a specific drug. In most of the clinical trials, PD-L1 expression has been assessed in tumor cells; however, atezolizumab's trials have also incorporated the determination of PD-L1 in immune cells. It is not possible to provide different samples of tissue in order to define the treatment that fits the best for just one single patient. It is extremely necessary that the regulatory agencies can take part of this issue in order that the pharmaceutical industry can define one universal assay to evaluate PD-L1 expression and can define similar cutoff points to be able to compare the different drugs for the same indication.

Beside PD-L1 expression other biomarkers are under investigation. Tumor heterogeneity and mutational density in lung cancer, and also the tumor microenvironment play a role in the variability of responses and outcomes in immunotherapy-treated patients regardless of the PD-L1 status. Probably PD-L1 expression is the first approach to define a biomarker that can predict response; however, it is insufficient to understand several mechanisms of resistance to drugs and also to understand why PD-L1 negative patients can achieve response to treatment.

Combining anti-PD-1s or anti-PD-L1s with anti-CTLA-4 drugs seems to be an interesting strategy to improve the outcomes in NSCLC. Clinical trials are already ongoing and preliminary reports are auspicious. Other strategies under development, related with immunotherapy in NSCLC, include combination of immunotherapy plus chemotherapy, antiangiogenics and specific-mutation targeted therapy (such as anti-EGFR or anti-ALK mutations). Immunotherapy is also under research in patients with local advanced disease as adjuvant treatment after chemo-radiation.

It is well known that the toxicity profile of immunotherapy is different from that of chemotherapy. Immunotherapy has a lower incidence of adverse events but it can be severe in some opportunities, hard to predict and with unusual forms of presentation. This scenario needs that oncologists have to be trained in immune-related adverse events recognition and their specific treatments [91].

Many of the NSCLC patients treated with immunotherapy worldwide have been able to access to these drugs because they have been enrolled in a clinical trial, or they have been supported in a compassionate use of a specific drug. However, the commercial value of these treatments is an issue that have ethical concerns. Indubitably, pharmaceutical companies make a big investment in drug's development, nevertheless, the current costs of the drugs will limit the possibility of the patients to be treated, and or will affect the economy of several countries in case of they were command to provide them by law. Even more, current combination of

immunotherapy treatments, if they are approved in future for NSCLC, could cost up to one million dollars per patient per year. This economical and ethical issue will force to select very well whom will be the patients that will have a real positive impact with immunotherapy treatment, and to look for biomarkers that can ensure in a correct manner a good and prolonged response to treatment.

In a short period of time, not only in NSCLC but also in several malignancies, immunotherapy became a mainstay of cancer treatment and it will likely help in the future to provide a powerful hand in cancer cure.

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