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Combination of immunotherapy with chemotherapy and radiotherapy in lung cancer: is this the beginning of the end for cancer?

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Abstract: Immune checkpoint inhibitors have significantly improved overall survival with an acceptable safety profile in a substantial proportion of non-small cell lung cancer (NSCLC) patients. However, not all patients are sensitive to immune checkpoint blockade and, in some cases, programmed death 1 (PD-1) or programmed death ligand 1 (PD-L1) inhibitors accelerate tumor progression. Several combination strategies are under evaluation, including the concomitant or sequential evaluation of chemotherapy or radiotherapy with immunotherapy. The current review provides an overview on the molecular rationale for the investigation of combinatorial approaches with chemotherapy or radiotherapy. Moreover, the results of completed clinical studies will be reported.

Keywords: chemotherapy, immune checkpoint inhibitors, immune evasion, immune resistance, non-small cell lung cancer, PD-1 inhibitors, PD-L1, PD-L1 inhibitors, radiotherapy

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Introduction

Lung cancer remains one of the leading causes of cancer-related deaths worldwide.[1] Non-small cell lung cancer (NSCLC) accounts for approximately 80–85% of all cases of lung cancer. Despite progress and the development of new drugs, the estimated 5-years overall survival (OS) remains just 16%.[1]

For a long time, platinum-based chemotherapy has been the main option for first-line treatment of metastatic NSCLC patients,[2–6] while docetaxel,[7] pemetrexed[8] and erlotinib[9] have been the standard therapy for the second-line setting.

Recently, the treatment landscape has evolved because of the introduction of agents targeting immune checkpoints, including programmed death 1 (PD-1) or programmed death ligand 1 (PD-L1). Immune checkpoint inhibitors have radically changed the treatment algorithm for metastatic NSCLC patients,[10] and significantly improved patients' prognosis. A proportion of patients enrolled in the phase I studies exploring the safety and activity of PD-1 or PD-L1 inhibitors experienced long-term survival. After a median follow up of 34 months, the survival curves reach a plateau between the second and the third year of treatment,[11] leading some investigators to introduce the concept of curative potential in the metastatic setting.

Preclinical findings showed that immunogenic cancer cells can be eliminated in an immunocompetent host. The genetic instability of cancer cells favors the development of immunogenic clones,[12,13] that are recognized by antigen-presenting cells (APCs) and dendritic cells, which stimulate the activation of CD8+ T-cells, which induce the killing of tumor cells. Inhibitory pathways that modulate and switch off the inflammatory response have evolved, in order to prevent the tissue damage derived from a prolonged activation of the immune system. Among these, the PD-1 axis dephoshorylates the T-cell receptor, induces T-cell apoptosis, decreases cytokine

production, and favors the immune evasion of cancer cells.[14] Agents targeting the PD-1 checkpoint disrupt this negative signaling, triggered by PD-L1/PD-L2, and restore T-cell antitumor function.

The two PD-1 inhibitors, nivolumab and pembrolizumab, and one PD-L1 inhibitor, atezolizumab, have been approved for the treatment of advanced NSCLC patients. Their development was initiated in pretreated patients, and then shifted to the first-line, neoadjuvant and adjuvant settings.

The current review provides an overview of the rationale for the design of clinical trials exploring the efficacy of combination strategies. Moreover, the available results will be reported.

Efficacy of monotherapy with PD-1 and PD-L1 inhibitors

Pembrolizumab is a humanized immunoglobulin (Ig)G4 PD-1 antibody, whose efficacy was explored in two phase III trials, the KEYNOTE-024[15] and the KEYNOTE-010,[16] designed in chemotherapy-naïve and pretreated advanced NSCLC patients, respectively (Table 1). A companion diagnostic test, the PD-L1 IHC 22C3 pharmDx, was developed and validated in the cohort of NSCLC patients receiving pembrolizumab in the phase I KEYNOTE-001 trial.[17] Patients with advanced NSCLC and a PD-L1 tumor proportion score of ⩾50%, stratified according to Eastern Cooperative Oncology Group performance status, histology and region of enrollment, were randomized between pembrolizumab or platinum-doublet chemotherapy in the KEYNOTE-024 study.[15] Cross-over to pembrolizumab was allowed in the chemotherapy arm at the time of progression. Pembrolizumab resulted in significantly longer progression-free survival (PFS), OS and objective response rate (ORR) compared with chemotherapy. The role of pembrolizumab in the second-line setting was evaluated in the KEYNOTE-010 trial, enrolling advanced previously-treated NSCLC patients, with PD-L1 expression in at least 1% of tumor cells. The study was designed to compare the efficacy of pembrolizumab with docetaxel in the intent to treat (ITT) population and in those cases with a PD-L1 tumor proportion score of ⩾50%.[16] Pembrolizumab significantly prolonged PFS in PD-L1 strong-positive cases only. However, significantly longer OS was observed under pembrolizumab both in all the patients who had undergone randomization, and in those with a tumor proportion score of more than 50%. Based on these findings, pembrolizumab was approved by the United States Food and Drug Administration (US FDA) in October 2015 in pretreated NSCLC patients with PD-L1 ⩾1%, and in December 2016 in chemotherapy-naïve patients expressing PD-L1 ⩾50%. In June 2016, pembrolizumab was approved by the European Medicines Agency in pretreated advanced NSCLC patients with PD-L1 ⩾1%, and on January 2017 in naïve patients with PD-L1 ⩾50%.

Similar to pembrolizumab, the efficacy of the human IgG4 PD-1 inhibitor nivolumab was investigated in chemotherapy-naïve and pretreated NSCLC patients. The CheckMate 026 trial compared nivolumab with platinum-based chemotherapy, in advanced naïve NSCLC patients expressing PD-L1 in at least 1% of tumor cells (Table 1).[18] The primary efficacy analysis population included patients with a PD-L1 expression level ⩾5%, while those with a PD-L1 expression level of 1% or more were considered for the secondary efficacy analysis population. Comparable PFS and OS between nivolumab and chemotherapy were found both in the ITT population and in the subgroup with PD-L1 ⩾5%. Two phase III trials, the CheckMate 017 (Table 1) and the CheckMate 057 (Table 1), were designed to demonstrate OS improvement of nivolumab over docetaxel in previously-treated patients with lung squamous cell carcinoma (LSCC)[19] and nonsquamous histology,[20] respectively. PD-L1 positivity did not represent an inclusion criterion, although PD-L1 status was retrospectively assessed in both trials. Nivolumab doubled ORR, prolonged PFS, reduced the risk of death by approximately 40%, and doubled OS at 1 year in LSCC cases.[19] The benefit was independent of PD-L1 status. In nonsquamous patients, nivolumab significantly prolonged OS, without increasing PFS, although the PFS rate at 1 year was higher in patients receiving nivolumab than docetaxel (19% and 8%, respectively), thus suggesting a delayed benefit in the nivolumab arm.[20] Based on these findings, nivolumab was approved by the US FDA in March 2015 for the treatment of patients with advanced LSCC, progressing on or after platinum-based chemotherapy, and in October 2015 the indication was expanded to include patients with nonsquamous NSCLC independent of PD-L1 expression.

Unlike nivolumab and pembrolizumab, atezolizumab is a humanized IgG1 monoclonal antibody

Table 1. Clinical trials exploring the efficacy of immune checkpoint inhibitors as monotherapy.

	Study	Line	Treatment comparison	PFS HR (95%CI)	OS HR (95%CI)
Pembrolizumab	KEYNOTE-024[15]	I	Platinum based chemotherapy	0.50 (0.37–0.68), p<0.001	0.60 (0.41–0.89), p=0.005
	KEYNOTE-010[16]	II	Docetaxel	PD-L1⩾50% 0.59 (0.44–0.78), p=0.0001 ITT 0.88 (0.74–1.05), p=0.07	PD-L1 ⩾ 50% 0.54 (0.38–0.77), p=0.0002 ITT 0.71 (0.58–0.88), p 0.0008
Nivolumab	CA-026[18]	I	Platinum based chemotherapy	1.15 (0.91–1.45), p=0.25	1.02 (0.80–1.30)
	CA-017[19]	II	Docetaxel	0.62 (0.47–0.81), p<0.001	0.59 (0.44–0.79), p<0.001
	CA-057[20]	II	Docetaxel	0.92 (0.77–1.11), p=0.39	0.73 (0.59–0.89), p=0.002
Atezolizumab	OAK[21]	II	Docetaxel	ITT 0.95 (0.82–1.10)	TC3 or IC3 0.41 (0.27–0.64), p<0.0001 ITT 0.73 (0.62–0.879), p=0.0003

CI, confidence interval; CT, chemotherapy; HR, hazard ratio; ITT, intent to treat; OS, overall survival; PFS, progression free survival.

targeting PD-L1. Its efficacy was compared with docetaxel in the phase III OAK trial,[21] in which patients were stratified by histology, number of previous lines of chemotherapy and PD-L1 expression, measured on tumor cells (TCs) and tumor infiltrating immune cells (ICs) (Table 1). The primary endpoint was the assessment of OS in the ITT population and according to PD-L1 levels. Prolonged survival was observed in the atezolizumab arm, regardless of PD-L1 expression levels on TC or IC, although the greatest advantage was seen in patients with high PD-L1 expression (TC3 or IC3). Prolonged PFS and improved ORR under atezolizumab were observed in strongly positive PD-L1 patients (TC3 or IC3) only. Based on these data, in October 2016 the US FDA approved atezolizumab for the treatment of patients with metastatic NSCLC, who have progressed during or following platinum-containing chemotherapy, independent of PD-L1 expression.

The discrepancy between the PFS and the OS benefit, observed in the KEYNOTE-010,[16] the CheckMate 057[20] and the OAK[21] trials, suggests that, in some patients, immune checkpoint inhibitors induce delayed anti-cancer immune effects, that impact on OS, despite RECIST progression. However, in case of tumor response, the tumor shrinkage is not delayed, and generally occurs after the first 2 months of treatment. Moreover, the analysis of the survival curves indicates that during the first 3 months, more patients in the immunotherapy arm develop progression, compared with those receiving docetaxel.[20] With the aim of improving the efficacy of immune checkpoint inhibitors, different strategies, including the concomitant or sequential use of PD-1/PD-L1 inhibitors with chemotherapy, radiotherapy or agents targeting other immune checkpoints, are currently under evaluation.

Combination strategies of chemotherapy and immune checkpoint inhibitors: rationale and results of clinical trials

In the past decades, chemotherapy was considered an immunosuppressive modality in the treatment of cancer. Accumulating evidence indicates the positive immunologic effects of chemotherapeutic agents.[22] Chemotherapy regulates the composition and function of tumor infiltrating lymphoid and myeloid cells,[23] and their presence influences patients' prognosis. Different molecular mechanisms have been identified, including the upregulation of nuclear factor kappa-light-chain-enhancer of activated B-cells (NF-B), the increase of CD8+ T-cells, a higher PD-L1 expression on TCs,[24] the maturation of APC, augmented tumor antigen presentation through the major

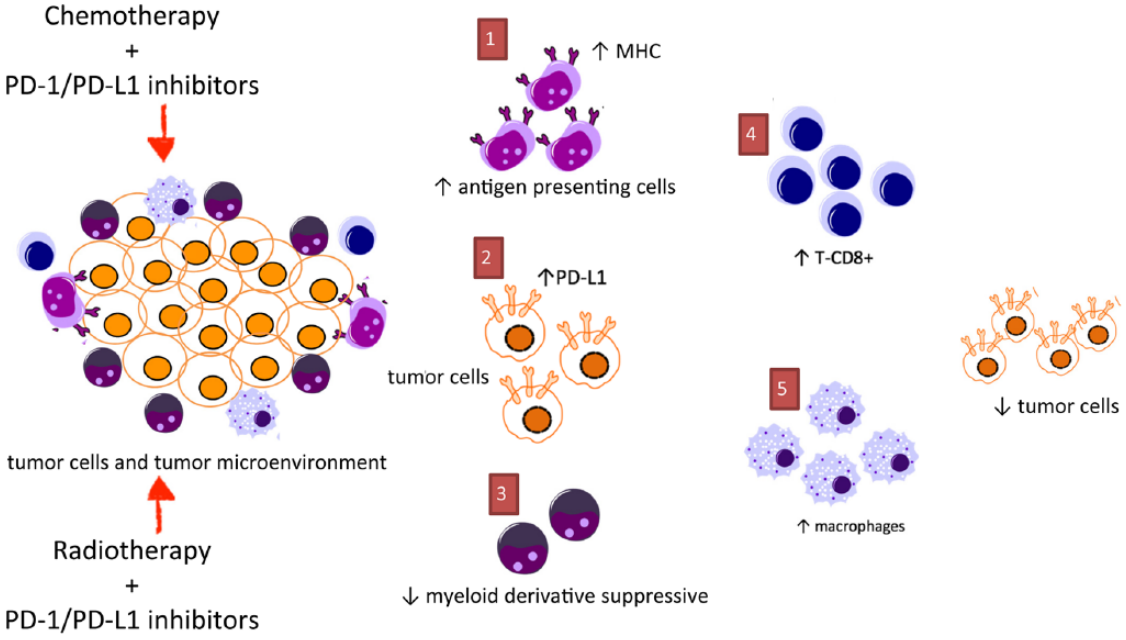

Figure 1. Effect of chemotherapy and radiotherapy on the modification of tumor microenvironment. Chemotherapy and radiotherapy favor the maturation of antigen-presenting cells and augmented tumor antigen presentation through the MHC class I, increased PD-L1 expression on tumor cells, downregulation of immunosuppressive cells at the tumor site (such as CD4+CD25+FOXP3+ Tregs or MDSCs), and the increase of CD8+ T-cells and macrophages. All of these effects augment the efficacy of immune checkpoint inhibitors on the reduction of tumor size.
MDSC, myeloid-derivative suppressive cells; MHC, major histocompatibility complex; PD-1, programmed death 1; PD-L1, programmed death ligand 1; Treg, regulatory T-cell.

histocompatibility complex (MHC) class I,[25] and downregulation of immunosuppressive cells at the tumor site [such as CD4+CD25+FOXP3+ regulatory T-cells (Tregs) or myeloid-derivative suppressive cells (MDSCs)][26] (Figure 1). It is currently unknown which signaling pathways are triggered by chemotherapy to modulate the immune system. In mice models, a relationship between NF-B signaling and MHC class I expression was observed.[27] The immunogenic changes differ according to the type of chemotherapy administered. In murine models of ovarian cancer under gemcitabine or paclitaxel, it was shown that gemcitabine decreased the number of MDSCs, while paclitaxel did not.[28]

Based on these findings, different clinical trials evaluating the safety and efficacy of platinum-based chemotherapy with immune checkpoint inhibitors have been designed.

The KEYNOTE-021 study was a phase I/II trial investigating the activity of pembrolizumab with carboplatin and paclitaxel, carboplatin, paclitaxel and bevacizumab, or carboplatin and pemetrexed (Table 2).[29] No significant adverse events were reported. With a median follow-up duration of 12 months, the ORR was 52%. Each cohort had a similar ORR regardless of the dose of pembrolizumab and the PD-L1 status. The association of carboplatin-pemetrexed-pembrolizumab was the most effective combination, with an ORR of approximately 70%. In order to further explore the activity of this combination, in the expansion part of the trial, treatment-naïve patients with advanced adenocarcinoma were randomized between carboplatin-pemetrexed or carboplatin-pemetrexed-pembrolizumab (Table 2).[30] Patients were enrolled independent of PD-L1 expression, even though tumor tissue was required at the time of enrollment, and PD-L1 was centrally analyzed. Cross-over to pembrolizumab was allowed in case of progression for patients enrolled in the chemotherapy arm. The addition of pembrolizumab almost doubled ORR, significantly prolonged PFS, decreased the median time to response, and significantly reduced the number of patients with progression at the first computed tomography

Table 2. Clinical trials exploring the efficacy of immune checkpoint inhibitors in combination with chemotherapy.

	Study	Phase	Chemotherapy associated	ORR	PFS HR (95%CI)
Pembrolizumab	KEYNOTE-021[29]	I/II	CBDCA+PTX CBDCA+PTX+BEVA CBDCA+PEM	52% 52% 70%	NA
	KEYNOTE-021[30]	II (expansion)	CBDCA+PEM	55% vs 29%	0.53 (0.31–0.91), p=0.010
Nivolumab	CA-012[31]	I	CDDP+GEM CDDP+PEM CBDCA+PTX	33% 47% 47%	NA
Atezolizumab	NCT01633970[32]	I	CBDCA+PEM	NA	8.4 months
Ipilimumab	NCT01285609[36]*	III	CBDCA+PTX (ipilimumab from 3-6 CT cycles)	44% vs 47%	0.87 (0.75–1.01)

BEVA, bevacizumab; CBDCA, carboplatin; CDDP, cisplatin; GEM, gemcitabine; ORR, objective response rate; PEM, pemetrexed; PFS, progression free survival; PTX, paclitaxel.

scan evaluation (two cases only). The efficacy was independent of PD-L1 expression. On 10 May 2017, the US FDA granted accelerated approval for pembrolizumab in combination with pemetrexed and carboplatin for the treatment of patients with previously untreated metastatic nonsquamous NSCLC. We are awaiting the results of the ongoing phase III KEYNOTE-189 [ClinicalTrials.gov identifier: NCT02578680] and KEYNOTE-407 [ClinicalTrials.gov identifier: NCT02775435] trials, which are exploring the efficacy of carboplatin or cisplatin and pemetrexed, and carboplatin-paclitaxel or nab-paclitaxel, with or without pembrolizumab in patients with nonsquamous and squamous NSCLC, respectively.

Similar to pembrolizumab, nivolumab was combined with platinum-based chemotherapy in the phase I CheckMate 012 trial (Table 2).[32] Patients with squamous histology received nivolumab plus gemcitabine-cisplatin, while those with nonsquamous histology received pemetrexed-cisplatin, and paclitaxel-carboplatin was used for both histologies. No dose-limiting toxicities were observed in the four cohorts. Results were promising, showing low frequencies of progressive disease, higher ORR and longer stable disease. Comparable results were found in patients with PD-L1 $\geqslant$ or $\leqslant$1%, suggesting that nivolumab may improve outcomes and extend survival of patients with advanced NSCLC in the first-line setting in combination with chemotherapy. The phase III CheckMate 227 trial (NCT02477826) is evaluating the benefit of adding nivolumab to platinum-doublet chemotherapy.

A phase Ib study tested the association of atezolizumab with different chemotherapy combinations. High response rate (64%) was achieved with no expected toxicity.[33] Preliminary results showed that patients under carboplatin-pemetrexed-atezolizumab reached the longest PFS and OS (PFS: 8.4 months; OS: 19.4 months) (Table 2). Overall, four phase III trials are investigating the combination of atezolizumab with different regimens, including first-line chemotherapy [ClinicalTrials.gov identifiers: NCT02366143, NCT02657434, NCT02367794 and NCT02367781].

The cytotoxic T-cell lymphocyte antigen-4 (CTLA4) inhibitor ipilimumab,[31] already approved in the treatment of melanoma, resulted in synergistic activity with chemotherapy in murine tumor models.[34] In a phase II study, NSCLC patients were randomized to receive ipilimumab in phased (from cycle 3 to 6 of chemotherapy) or concurrent (in cycles 1 to 4 of six chemotherapy cycles) with paclitaxel and carboplatin, followed by ipilimumab maintenance.[35] Ipilimumab, when administered in phased, significantly prolonged PFS. The greatest improvement was registered in patients with squamous NSCLC. These results formed the bases for the

design of a randomized, double-blind, phase III trial exploring ipilimumab, administered in a phased regimen, with carboplatin and paclitaxel in patients with squamous NSCLC (Table 2).[36] The primary endpoint was the assessment of OS. No significant difference in terms of OS, PFS or ORR were found between the two treatment arms. The divergent findings observed with PD-1/PD-L1 inhibitors and ipilimumab might be partially due to the different mechanisms of function of CTLA-4 inhibitors and agents targeting the PD-1 axis. While ipilimumab stimulates early-stage T-cell activation in the lymphoid compartment, nivolumab, pembrolizumab and atezolizumab activate T-cell function within the tumor microenvironment. It is possible that ipilimumab does not generate a sufficient antitumor response. Recently, two immune checkpoint inhibitors, the PD-L1 inhibitor durvalumab and the CTLA-4 inhibitor tremelimumab, were combined with cisplatin-pemetrexed in a phase Ib study.[37] The preliminary results showed an ORR of approximately 50%, without an increase in the frequency of adverse events, that in the majority of cases were of grade 2, suggesting that combining two immune checkpoint inhibitors with standard chemotherapy is well tolerated.

Combination strategies of radiotherapy and immune checkpoint inhibitors: rationale and results

Preclinical studies have found synergistic activity between radiation and PD-1 inhibition. In Kras-driven genetically engineered mouse models of NSCLC, radiotherapy combined with an anti-PD-1 agent induced long-lasting tumor regressions.[38] Moreover, the upregulation of PD-L1 was observed 24 h after radiotherapy. In 1953, the abscopal effect was described for the first time. It refers to the effect of local radiation at a distant nonirradiated volume, suggesting that local radiotherapy might favor the release of tumor-associated antigens, which stimulate a systemic immune response.[39–41] Similar to the effect driven by chemotherapy, radiotherapy decreases the number of Tregs, upregulates MHC class I molecules on cancer cells, induces immunogenic cell death, which induces the release of antigens and damage-associated molecular patterns, which in turn stimulate the recruitment and maturation of dendritic cells and promotes antigen presentation (Figure 1).[42–44] Thanks to these effects on immune modulation, radiotherapy might favor the conversion of nonimmunogenic tumors into immunogenic ones. Clinically relevant questions regarding the optimal fractionation schedule, the total radiation dose, the clinical target volume, the safety, and the timing, are objectives of ongoing research. Moreover, considering that the tumor microenvironment differs within the different organs, the systemic tumor immune response might be influenced by the site chosen to perform radiotherapy. In preclinical models, the concurrent administration of immune checkpoint inhibitors and radiotherapy was superior to the sequential strategy, and hypofractionated or stereotactic radiotherapy enhanced immunogenicity.[45,46]

Due to the exclusion criteria, a limited number of patients enrolled in the phase III trials evaluating the efficacy of immune checkpoint inhibitors received radiotherapy. A total of 97 NSCLC patients enrolled in the phase I KEYNOTE-001 study[17] received radiation before the initiation of pembrolizumab.[47] In the majority of cases, radiotherapy was administered with a palliative intent, and almost half underwent thoracic radiation. Significantly longer PFS and OS were found in patients receiving radiotherapy. The greatest advantage was registered in the subgroup treated with extracranial radiation therapy. However, a higher frequency of pulmonary toxicity was observed in those patients who received previous thoracic radiotherapy compared with those who had not, despite no statistical difference being observed in terms of grade 3 pneumonitis.[47]

Several multi-cohort phase I/II studies enrolling patients with different histologies are currently ongoing. Their primary end points include safety, ORR, the evaluation of the maximum tolerated dose (MTD) or of the dose of radiation to be delivered, the PFS, or changes in PD-L1 expression levels. In some trials stereotactic ablative radiotherapy is used, while in others hypofractionated radiation therapy is being tested (Table 3).

Another group of patients who might benefit from the addition of immunotherapy following chemotherapy and radiotherapy are those with locally advanced resectable or unresectable lung tumors, who require a multimodality strategy. Resectable patients, with the involvement of the hilar lymph nodes, generally receive adjuvant platinum-based chemotherapy,[48] while postoperative radiotherapy and chemotherapy represent the standard treatment for patients with N2 lymph node

Table 3. Ongoing clinical trials of immune checkpoint inhibitors in combination with radiotherapy.

	Study	Phase	Radiotherapy associated	Primary endpoints
Pembrolizumab	NCT02587455	I	Palliative EBRT	Toxicity
	NCT02621398	I	IMRT or 3D-CRT before or after pembrolizumab	Toxicity
	NCT02303990	I	Hypofractionated radiotherapy	AE
	NCT02608385	I	SBRT	Optimal dose (SBRT)
Nivolumab	NCT02696993	I/II	SBRT (1 fraction) or WBRT (3 Gy 10 fractions)	Optimal dose
	NCT02434081	II	Thoracic EBRT	AE
	NCT02831933	II	SBRT, 6 Gy 5 fractions before nivolumab	RR

3D-CRT, 3-dimensional conformal radiation therapy; AE, adverse event; EBRT, external beam radiation therapy; IMRT, intensity-modulated radiation therapy; RR, response rate; SBRT, stereotactic body radiation therapy; WBRT, whole brain radiation therapy.

involvement.[49] Adjuvant chemotherapy determines an absolute OS benefit of 3.9% and 5.4% at 3 and 5 years, respectively.[50] Different studies, currently ongoing, are exploring the benefit of PD-1 and PD-L1 inhibitors following adjuvant chemotherapy.

Concurrent or sequential chemoradiation is the treatment strategy used for patients with unresectable locally advanced NSCLC.[51] In this subgroup, survival rates remain poor, with only 15% of patients still alive at 5 years. With the aim of improving clinical outcome after chemoradiotherapy, the phase III PACIFIC study was designed, to compare the IgG1 PD-L1 inhibitor durvalumab with placebo, in patients with stage III unresectable NSCLC, not progressing after platinum-based chemoradiotherapy.[52] Co-primary endpoints were PFS and OS. A total of 713 patients, stratified according to age (<65 *versus* $\geqslant 65$ years), sex, and smoking history, were randomized in a 2:1 ratio between durvalumab and placebo within 1–42 days after chemoradiotherapy. Median PFS was significantly longer in patients receiving durvalumab, with a reduction in the risk of progression of 48%, and a three-fold increase in median PFS. The benefit was independent of PD-L1 status, which was assessed on tumor tissue biopsied before the beginning of chemoradiotherapy. We do not know the impact of chemoradiation in potentially changing PD-L1 expression, increasing release of tumor-associated antigens, decreasing number of Tregs, and augmenting activation of the immune system. More patients in the placebo group had distant relapses compared with patients treated with durvalumab. Moreover, a reduction in the frequency of brain recurrences was observed with durvalumab. The ORR was significantly higher in patients receiving durvalumab, with a longer median duration of response. Although the incidences of pneumonitis or radiation pneumonitis were increased with both durvalumab and placebo, a higher frequency of low-grade pulmonary toxicity was observed in those patients who received previous thoracic radiotherapy and durvalumab compared with those who had not, with no statistical difference observed in terms of grade 3 pneumonitis.[52] Based on these results, on 31 July 2017 the US FDA granted breakthrough therapy designation of durvalumab for patients with locally advanced unresectable NSCLC, not progressing following platinum-based chemoradiation therapy.

Comments and future perspectives

First-line platinum-based chemotherapy has been for years the cornerstone of treatment of advanced NSCLC patients. The recent introduction of immune checkpoint inhibitors targeting the PD-1–PD-L1 axis has radically changed the treatment algorithm in the first- and second-line settings. Moreover, the preclinical and clinical findings about the efficacy of combination strategies, including chemotherapy, radiotherapy and immune checkpoint inhibitors, have further improved the therapeutic opportunities, and increased the complexity of therapeutic selection. Currently, the most remarkable challenge remains the lack of predictive biomarkers able to identify

which patients might gain most benefit from immune checkpoint inhibitors, which patients may develop tumor flare under PD-1 and PD-L1 blockade, or which patients might benefit from a combinatorial approach.

Retrospective and prospective studies have evaluated the predictive role of PD-L1 expression, analyzed by immunohistochemistry (IHC). PD-L1 strong-positive patients are more likely to benefit from immune checkpoint inhibitors.[16,20,21] However, phase III trials have demonstrated that PD-L1-negative patients might also benefit in terms of OS from PD-1/PD-L1 inhibitors.[16,20,21] One explanation for these findings might be related to the fact that patients were not rebiopsied before entering in the clinical trials investigating the efficacy of PD-1 blockade in the second- or third-line settings. PD-L1 expression was evaluated on the tumor biopsy performed at the time of initial diagnosis. A growing body of evidence suggests that PD-L1 is a dynamic marker that might change over the course of chemotherapy or radiotherapy. A switch from PD-L1-negative to PD-L1-positive expression could have occurred in some cases. Overall, four IHC assays have been tested in clinical trials: the Dako 22C3 pharmDx, developed in association with pembrolizumab; the 28-8 pharmDx and the SP 142, approved by the US FDA as complementary diagnostics, and associated with nivolumab and atezolizumab, respectively; and the Ventana SP263, developed in combination with durvalumab. Recently, the Blueprint PD-L1 IHC Assay Comparison Project has been designed to compare the four PD-L1 IHC assays.[53] The aim was not to define the most sensitive or specific assay, but to build an algorithm to be used in clinical practice. Tissue from 38 surgically resected NSCLC patients was analyzed with the four antibodies in the phase I part of the study. A total of 50% of cases were classified as positive by all of the antibodies, in 13% of patients the PD-L1 expression was below the cutoff by all of the assays, while discordant results were observed in 37% of cases.[53] The phase II part of the study is currently ongoing in a larger number of patients, including small biopsies and cytological specimens from patients receiving immune checkpoint inhibitors. Besides PD-L1 status, other markers have been proposed as predictive of response to immunotherapy. Recent studies have demonstrated that tumors with a high mutational burden, abundant neoantigens and microsatellite (MSI)-high status are associated with a good response to anti-PD-1/PD-L1 therapy.[54–56] Exome sequencing, performed in two independent cohorts of NSCLC patients receiving pembrolizumab,[56] revealed that a high number of nonsynonymous mutations is associated with clinical benefit, higher ORR and longer PFS.

Another remarkable question pertains to the identification of those patients who do not benefit from PD-1/PD-L1 blockade. Retrospective analyses including patients with solid tumors enrolled in phase I trials with immune checkpoint inhibitors documented hyperprogression in approximately 9% of cases.[57] Hyperprogressors were defined as those patients in which the tumor growth rate, observed during the 8 weeks following the beginning of immunotherapy, was at least two-fold greater compared with that observed during the 8 weeks before the initiation of PD-1/PD-L1 inhibition. The presence of amplification in the mouse double minute 2 homolog (*MDM2*) gene was identified by another group as a common biologic feature in patients developing hyperprogression under immunotherapy, independent of tumor histology.[58] PD-1/PD-L1 inhibition favors the release of interferon-gamma (IFN-γ), which activates the janus kinase-STAT (JAK-STAT) signaling pathway, which stimulates interferon regulatory factor 8 (IRF-8). The binding between IRF-8 and the *MDM2* promoter, in the presence of *MDM2* amplification, determines *MDM2* hyperexpression, which negatively regulates p53 activity through the induction of p53 degradation.[58] According to this hypothesis, patients carrying *MDM2* amplification might develop a tumor flare while receiving immune checkpoint inhibitors.

Data from the literature indicate that the genomic context in which tumors arise influences the tumor microenvironment, and this impacts on the efficacy of drugs targeting immune checkpoints. The loss of phosphatase and tensin homolog (*PTEN*) gene in patients with melanoma is associated with a reduction in the percentage of CD8+ T-cells at the tumor site, thus determining immune resistance.[59] This is similar to what has been observed in patients with melanoma harboring gain-of-function mutations in the β-catenin gene, or loss-of-function mutations in adenomatous polyposis coli, *AXIN1*, or transcription factor 1 (*TCF1*) genes.[60] These molecular alterations are associated with active β-catenin signaling, which prevents the transcription of a T-cell gene expression signature, resulting in a non-T-cell inflamed phenotype.

Our knowledge on cancer immunology is not fully complete. Next-generation sequencing might help in the future to better molecularly classify patients, define the link between tumor genetics and tumor microenvironment, and identify those patients who derive benefit from immune checkpoint inhibitors as monotherapy, or those who require combination strategies. Another issue regards the identification of the molecular mechanisms responsible for acquired resistance to immunotherapy after an initial tumor regression.[61] The understanding of these acquired resistance mechanisms might help in the definition of combinatorial strategies in case of PD-1 or PD-L1 inhibitor relapse, favoring the development of an algorithm of sequential therapy.

Even though the PACIFIC study has demonstrated the clear advantage of the addition of durvalumab following chemoradiation, concerns about the risks of pneumonitis exist, and the identification of those patients who really benefit from the addition of durvalumab is warranted.

Conclusion

In the next years, PD-1 and PD-L1 inhibitors alone or in combination with chemotherapy, radiotherapy or other immune checkpoint inhibitors will become the standard of care for the first-line treatment of NSCLC patients whose disease is not driven by a genetic alteration. Different trials are exploring the advantage of immune checkpoint inhibitors in the neoadjuvant and adjuvant settings. In this scenario, clinicians will deal with new challenges, and the complexity of treatment decision-making will be further increased. Results from the ongoing phase III trials that are investigating the combination of immunotherapy with chemotherapy regimens will better establish the efficacy and toxicity of this approach. The understanding of the evolving immunological tumor profile will become more relevant, and crucial to define therapeutic algorithms, who are those patients who need to receive a combination strategy, or which is the sequential treatment algorithm to use, or how to combine chemotherapy, radiotherapy or immunotherapy. It is also possible that other combination strategies will emerge, and that thanks to the extended use of these drugs, cancer will be transformed into a chronic disease.

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The authors declare that there is no conflict of interest.

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