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MINIREVIEWS

Use of programmed cell death protein ligand 1 assay to predict the outcomes of non-small cell lung cancer patients treated with immune checkpoint inhibitors

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Abstract

The recent discovery of immune checkpoints inhibitors, especially anti-programmed cell death protein 1 (PD-1) and anti-programmed cell death protein ligand 1 (PD-L1) monoclonal antibodies, has opened new scenarios in the management of non-small cell lung cancer (NSCLC) and this new class of drugs has achieved a rapid development in the treatment of this disease. However, considering the costs of these drugs and the fact that only a subset of patients experience long-term disease control, the identification of predictive biomarkers for the selection of candidates suitable for treatment has become a priority. The research focused mainly on the expression of the PD-L1 receptor on both tumor cells and/or immune infiltrates determined by immunohistochemistry (IHC). However, different checkpoint inhibitors were tested, different IHC assays were used, different targets were considered (tumor cells, immune infiltrates or both) and different expression thresholds were employed in clinical trials. In some trials the assay was used prospectively to select the patients, while in other trials it was evaluated retrospectively. Some confusion emerges, which makes it difficult to easily compare the literature data and to translate them in practice management. This mini-review shows the possibilities and pitfalls of the PD-L1 expression to predict the activity and efficacy of anti PD1/PD-L1 monoclonal antibodies in the treatment of NSCLC.

Key words: Predictive biomarkers; Immunotherapy; Checkpoint inhibitors; Programmed cell death protein ligand 1; Non-small cell lung cancer

Core tip: Use of programmed cell death protein ligand 1 (PD-L1) assay to predict the outcomes of non-small cell lung cancer (NSCLC) patients treated with immune checkpoint inhibitors. This minireview underlines promises and pitfalls of the PD-L1 expression to predict the activity and efficacy of programmed cell death protein 1/PD-L1 inhibitors in NSCLC.

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INTRODUCTION

Lung cancer is the leading cause of cancer-related deaths worldwide. Non-small cell lung cancer (NSCLC) accounts for more than 85% of primary lung cancers. Approximately two-thirds of NSCLC patients are diagnosed at an advanced stage and their prognosis remains poor[1].

The discovery of driver oncogene alterations such as epidermal growth factor receptor (EGFR) mutations, anaplastic lymphoma kinase (ALK) rearrangements, and identification of their targeted inhibitors, have dramatically improved the outcomes in highly selected patients[2,3]. Conversely, the last generation chemotherapy regimens date back more than 15 years and, unfortunately, the clinical results obtained with this strategy have reached a plateau.

The recent improvements in the knowledge of cancer immunoediting and the discovery of immune checkpoint inhibitors have led to new opportunities in the treatment of NSCLC and have paved the way to improve the outcomes for a considerable number of patients[4-6]. The immunoresponse, driven by T-lymphocytes, is regulated by a complicated balance between inhibitory checkpoints and activating signals. Some key immune checkpoint proteins have been identified: Cytotoxic T-lymphocytes antigen 4 (CTLA-4) and programmed death-1 (PD-1). In the priming phase, which occurs in lymph-nodes, the CTLA-4 receptor, located on the surface of the lymphocyte T cells binds the B7-receptor on the cellular membrane of the dendritic cell. In the effector phase, which occurs peripherally, the PD-1 located on the cellular membrane of lymphocyte T cells, binds programmed cell death protein ligand 1 (PD-L1) and PD-L2, which are expressed by tumor cells, stromal cells, or both. These observations have led to the development of a monoclonal antibody-directed against CTLA4 and PD1/PD-L1 proteins such as ipilimumab (anti-CTLA-4), nivolumab and pembrolizumab (anti PD1), atezolizumab, durvalumab, avelumab (anti-PD-L1). These new classes of drugs have gained a rising development in the treatment of NSCLC: So far, nivolumab, pembrolizumab and atezolizumab have been approved by the Food and Drug Administration for second-line treatment of advanced NSCLC. In this setting, all the above-mentioned drugs have shown a clear superiority in terms of activity and efficacy compared to standard chemotherapy. However, although well tolerated, these new drugs are highly effective only in a limited subset of patients; this fact, together with the high economic impact, has evidenced the need to identify of biomarkers able to select patients with the highest likelihood of benefit[7]. The attention of researchers and clinicians has focused mainly on the expression of PD-L1 on tumor cells and/or immune infiltrates determined by immunohistochemistry (IHC), since this protein seems to be critical in the PD-1/PDL-1 pathway. Unfortunately, the heterogeneity of tests, targets and scores has produced conflicting results in the literature.

ANTI-PD-1 ANTIBODIES

Nivolumab beyond first-line treatment

In a pivotal phase Ⅰ study published by Gettinger *et al*[8], 296 patients with advanced solid tumors, including 122 NSCLCs, were treated with an escalating dose of anti PD-1 antibody (BMS-936558). PD-L1 expression was evaluated by using a non-commercial anti PD-L1 monoclonal antibody (5H1) in formalin-fixed tumor specimens and fresh tumor tissues. Positivity was defined as ⩾ 5% tumor cell membrane staining in a minimum number of 100 evaluable cells. PD-L1 expression was retrospectively evaluated in 10 patients: None of the 5 patients with negative tumors had an objective response whereas 1 out of 5 patients bearing positive tumors responded to treatment. This phase Ⅰ trial has been recently updated by recruiting an additional 129 patients who reported an overall response rate of 17%. A total of 68 samples were retrospectively tested for PD-L1 expression: Patients with positive tumors achieved an overall response rate of 15%, a median progression free survival (mPFS) of 3.3 mo (95%CI: 1.8-7.5), and a median overall survival (mOS) of 7.8 mo (95%CI: 5.6-21.7). Patients with negative tumors achieved an objective response rate of 14%, an mPFS of 1.8 mo (95%CI: 1.7-2.3), and a mOS of 10.5 mo (95%CI: 5.2-14.8). Responses were obtained regardless of histology (squamous or non-squamous), EGFR and KRAS status, PD-L1 positivity or negativity. Conversely, a smoking history seemed to be an interesting parameter: patients smoking more than 5 pack-years did much better (overall response rate of 30% *vs* 0% for < 5 pack-years). One intriguing observation, subsequently confirmed, was that some patients, who discontinued therapy for toxicity, maintained clinical remission in the absence of more than 9 months' treatment (Table 1).

In the CheckMate 063 multicenter phase Ⅱ study the nivolumab 3 mg/kg q 14 activity was evaluated in heavily pre-treated advanced squamous cell carcinoma of the lung[9]. The patient population was highly refractory to chemotherapy, with almost two-thirds having previously received three or more systemic treatments. A total of 117 patients were enrolled: The overall response rate, evaluated by an independent radiology review Committee, was 14.5% (95%CI: 8.7-22.2). Seventy-six tumors were retrospectively assessed for PD-L1 expression on formalin-fixed, paraffin-embedded (FFPE)

Table 1 Correlation between nivolumab activity and outcome and programmed cell death protein ligand 1 immunohistochemestry score

Author/study	Marker antibody	Tumor type	Treatment line	PD-L1 cutoff	N pts	Response (%)	mPFS mo (95%CI)	mOS mo (95%CI)
Nivolumab								
Gettinger *et al*[8]	Dako 28-8	NSCLC	> 2	⩾ 5 %	33	15	3.3 (1.8-7.5)	7.8 (5.6-21.7)
Phase Ⅰ				< 5%	35	14	1.8 (1.7-2.3)	10.5 (5.2-14.8)
Rizvi *et al*[9] CM 063	Dako 28-8	Squamous	⩾ 2	⩾ 5%	25	24	NR	NR
Phase Ⅱ		NSCLC		< 5%	51	14	NR	NR
Brahmer *et al*[10]	Dako 28-8	Squamous	> 1	⩾ 10%	36	19	3.7 (NR)	11 (NR)
CM 017		NSCLC		< 10%	81	16	2.3 (NR)	8.2 (NR)
Phase Ⅲ				⩾ 5%	42	21	4.8 (NR)	10 (NR)
				< 5%	75	15	2.2 (NR)	8.5 (NR)
				⩾ 1%	63	17	3.3 (NR)	9.3 (NR)
				< 1%	54	17	3.1 (NR)	8.7 (NR)
Borgheai *et al*[11]	Dako 28-8	Non squamous	> 1	⩾ 10%	86	37	5.0 (NR)	19.9 (NR)
CM 057		NSCLC		< 10%	145	11	2.1 (NR)	9.9 (NR)
Phase Ⅲ				⩾ 5%	95	34	5.0 (NR)	19.4 (NR)
				< 5%	136	14	2.1 (NR)	9.8 (NR)
				⩾ 1%	123	31	4.2 (NR)	17.7 (NR)
				< 1%	108	9	2.1 (NR)	10.5 (NR)
Gettinger *et al*[12]	Dako 28-8	NSCLC	1	⩾ 50%	12	50	NR	NR
CM 012				< 50%	34	15	NR	NR
Phase Ⅰ				⩾ 25%	18	44	NR	NR
				< 25%	28	11	NR	NR
				⩾ 10%	20	40	NR	NR
				< 10%	26	12	NR	NR
				⩾ 5%	26	31	NR	NR
				< 5%	20	15	NR	NR
				⩾ 1%	32	28	NR	NR
				< 1%	14	14	NR	NR
Rizvi *et al*[13] CM012	Dako 28-8	NSCLC	1	⩾ 1%	23	48	6.0 (< 0.1+- 21.8)	20.2 (6.2-28.8+)
Phase Ⅰ				< 1%	21	43	5.2 (0.9+-28.7+)	19.2 (4.5-29.7+)
Socinski *et al*[14]	Dako 28-8	NSCLC	1	⩾ 5%	NR	76.80	NR	NR
CM 026				< 5%	NR	NR	NR	NR
Phase Ⅲ				⩾ 25%	NR	48.70	NR	NR
				< 25%	NR	NR	NR	NR
				⩾ 50%	NR	32.50	NR	NR
				< 50%	NR	NR	NR	NR
				⩾ 75%	NR	20.70	NR	NR
				< 75%	NR	NR	NR	NR

CM: CheckMate; NR: Not reported; pts: Patients; NSCLC: Non-small cell lung cancer; PD-L1: Programmed cell death protein ligand 1; mPFS: Median progression free survival; mOS: Median overall survival.

specimens with a commercially validated, automated immunohistochemical assay (Dako, Carpinteria, CA, United States) by using a 28-8 clone (rabbit anti-human PD-L1) with a 5% expression threshold to define PD-L1 positivity. Response rates were 24% and 14% in patients with positive *vs* negative tumors respectively (Table 1).

In the CheckMate 017 phase Ⅲ trial a total of 272 pre-treated patients with advanced squamous lung tumors were randomized to receive 3 mg/kg of nivolumab every 2 wk or 75 mg/m^2 of docetaxel every 3 wk. The primary end-point was overall survival OS[10]. This pivotal trial demonstrated a statistically and clinically significant survival advantage in favor of immunotherapy with a reduction in risk death of 41% [hazard ratio (HR) = 0.59, 95%CI: 0.44 to 0.79, P < 0.001]. The mOS was 9.2 mo (95%CI: 7.3 to 13.3) for nivolumab *vs* 6.0 mo (95%CI: 5.1 to 7.3) for docetaxel and the response rates were 20% and 9% respectively (P = 0.0008). PD-L1 protein expression was retrospectively evaluated in pretreatment tumor-biopsies with the Dako assay and the response rate was compared at pre-specified expression levels of 1%, 5% or 10%. The response rate was 17% in tumours with PD-L1 positivity ⩾ 1%; this rate of response was indistinguishable from that observed in PD-L1 negative specimens (< 1%). The response rate was 21% in tumors with PDL-1 positivity ⩾ 5% and 15% in tumors with PD-L1 < 5%. Ultimately, the response rates were 19% and 16% in PD-L1 positive tumors ⩾ 10% or < 10%, respectively (Table 1). It is noteworthy that the benefit of OS in this study was independent of the PD-L1 scores.

In the CheckMate 057 randomized phase Ⅲ trial, 582 pretreated advanced non squamous NSCLC patients received 3 mg/kg of nivolumab every 2 wk or 75 mg/m^2 of docetaxel every 3 wk[11]. Also in this study, the primary end-point was OS; mOS in the nivolumab arm was significantly longer than in the docetaxel arm, 12.2 mo *vs* 9.4 mo, respectively; the overall

response rates were 19% with nivolumab and 12% with docetaxel. The PD-L1 protein was retrospectively assessed with the Dako assay in pre-treatment archival or recent tumor-biopsy specimens. The response rate was compared at pre-specified expression levels of 1%, 5% and 10%. The response rate was 31% and 9% in tumors with PD-L1 positivity ⩾ 1% or < 1% respectively; the response rate was 36% and 10% in PD-L1 positive tumors ⩾ 5% or < 5%, and the response rate was 37% or 11% in PD-L1 positive tumors ⩾ 10% or < 10% respectively (Table 1).

Nivolumab for first-line treatment

In the CheckMate 012 study 52 treatment-naive advanced NSCLC patients received nivolumab at the dose of 3 mg/kg every 2 wk[12]. The response rate was 23% and the efficacy data were very encouraging: mPFS was 3.6 mo and mOS was 19.4 mo. On the whole, tumor shrinkage was obtained independently of the PD-L1 expression; however, the greater the PD-L1 positivity increase, the higher the probability of response. Conversely, there was no clear association between mPFS and mOS and PDL-1 expression (Table 1).

In the Rizvi *et al*[13]'s trial, patients with advanced NSCLC received 10 mg/kg of nivolumab every 2 wk in combination with cisplatin plus gemcitabine or pemetrexed or carboplatin plus paclitaxel; or, they received 5 mg/kg of nivolumab 5 mg/kg every 2 wk with carboplatin plus paclitaxel. The response rates were 33% in the nivolumab plus cisplatin/gemcitabine group, 47% in the cisplatin plus pemetrexed group, 47% in the carboplatin and paclitaxel group and 43% in the nivolumab 5 mg/kg plus carboplatin/paclitaxel group. In patients with PDL-1 expression ⩾ 1%, the response rate was 48%, whereas in patients with PD-1 < 1% the response rate was 43%. No relationship was observed between PDL-1 expression and mPFS and mOS (Table 1).

In the CheckMate 026 phase Ⅲ trial, patients with untreated advanced NSCLC and PD-L1 tumor positivity > 1% were randomized to receive 3 mg/kg IV of nivolumab 3 mg/kg IV every 2 wk or platinum-based chemotherapy every 3 wk for 6 cycles[14]. The primary end-point of the study was to demonstrate an improved PFS for patients with PD-L1 tumor-expression ⩾ 5%. Median PFS was 4.2 and 5.9 mo with nivolumab and platinum-based chemotherapy, respectively (HR = 1.15, 95%CI: 0.91-1.45, P = 0.25). Median OS was 14.4 mo for immunotherapy and 13.2 mo for chemotherapy. The preliminary results of this study presented at the ESMO meeting showed that the PD-L1 score did not predict the response rate (Table 1).

Pembrolizumab beyond first-line treatment

KEYNOTE-001 was a large phase Ⅰ study with an NSCLC expansion cohort including a total of 495 advanced NSCLC patients who received 2 mg or 10 mg/kg of pembrolizumab every 3 wk or 10 mg/kg every 2 wk[15]. One hundred and eighty-two patients were assigned to the "training group" recruited to define the PD-L1 positivity threshold on pre-treatment tumor biopsy (using the antibody clone 22C3-Dako-IHC assay). The remaining 313 patients were treated in the "validation group". According to the data obtained from the training group, a PD-L1 tumor expression of 50% was identified as threshold of positivity. The validation group patients with a tumor PD-L1 score ⩾ 50% had a response rate of 45.2% (95%CI: 33.5-57.3): This figure was 17% (95%CI: 9.9-25.1) in patients with a score 1%-49% and 3% (95%CI: 2.3-28.2) in patients with PD-L1 < 1% (Table 2). Noteworthy, a deterioration of the PD-L1 antigen was observed in tumor samples sectioned more than 6 mo before staining. The response rates were higher in former or current smokers compared to non-smokers (22.5% *vs* 10.3%). Treatment was effective at all tested doses and schedules, therefore an every-3-wk schedule was chosen for the phase Ⅲ study.

These data were confirmed in a large prospective randomized phase Ⅱ/Ⅲ trial (KEYNOTE-010). This study enrolled 1034 previously treated PD-L1-positive NSCLC patients (PD-L1 expression ⩾ 1% of tumour cells) and compared 2 mg or 10 mg/kg pembrolizumab every 3 wk *vs* 75 mg/m^2 docetaxel every 3 wk in terms of OS and PFS[16]. PD-L1 expression was evaluated in the archival tumor samples of 456 patients, while new biopsy material was collected before a study entry for the remaining patients. No differences in mPFS emerged between immunotherapy and chemotherapy. Overall survival was significantly longer in both pembrolizumab arms compared to the docetaxel arm: The HRs were 0.71 (95%CI: 0.58-0.88, P = 0.0008) and 0.61 (95%CI: 0.49-0.75, P < 0.0001) respectively for the two dose-levels of pembrolizumab. However, in patients with PD-L1 positivity ⩾ 50% the HRS for OS were 0.54 (P = 0.0002) in the pembrolizumab 2 mg/kg arm and 0.50 (P ⩽ 0.0001) in the Pembrolizumab 10 mg/kg treatment arm respectively; in addition, in this PD-L1 selected subgroup of patients also PFS was significantly longer than with chemotherapy (Table 2). In the total population, the response rates were 18% with pembrolizumab and 9% with docetaxel; in patients with PD-L1 positivity ⩾ 50% the response rate was about 30%, while it was 8% in patients with tumors showing a PD-L1 expression level < 50% (Table 2). Consistent with the results from the nivolumab trials, pembrolizumab was more tolerable than docetaxel and did significantly better in both squamous and non-squamous histology. Similarly, patients with EGFR mutated tumors seemed to have no survival advantage with immunotherapy over chemotherapy despite the small number of patients.

Pembrolizumab in first-line treatment

The KEYNOTE-024 was a phase Ⅲ trial in which 350 untreated NSCLC patients with a PDL-1 tumor

Table 2 Correlation between pembrolizumab activity and outcome and programmed cell death protein ligand 1 immunohistochemestry score

Author/study	Marker antibody	Tumor type	Treatment line	PD-L1 cutoff	N pts	Response	mPFS mo (95%CI)	mOS mo (95%CI)
Pembrolizumab								
Garon *et al*[15] KN001	Dako 22C3	NSCLC	⩾ 1	⩾ 50%	73	45.20%	6.4 (4.2-NR)	NR (NR-NR)
				1%-49%	103	17%	4.1 (2.3-4.4)	10.6 (7.3-NR)
				< 1%	28	3%	4 (2.1-6.2)	10.4 (5.8-NR)
Herbst *et al*[16] KN010	Dako 22C3	NSCLC	⩾ 2	⩾ 50%	290	30%	14.9 (10.4-NR)	5.0 (4.0-6.9)
				1%-49%	400	10%	17.3 (11.8-NR)	5.2 (4.1-8.1)
Reck *et al*[17] KN024 Phase Ⅲ	Dako 22C3	NSCLC	1	⩾ 50%	305	44.80%	10.3 (6.7-NR)	NA
Langer *et al*[18] KN021 Phase Ⅲ	Dako 22C3	Non squamous NSCLC	1	⩾ 50%	20	80%	13 (8.3-NR)	NA
				1%-49%	19	29%		
				< 1%	21	57%		

KN: KeyNote; NR: not reported; pts: Patients; NA: Not available; NSCLC: Non-small cell lung cancer; PD-L1: Programmed cell death protein ligand 1; mPFS: Median progression free survival; mOS: Median overall survival.

score of 50% or greater were randomized to receive pembrolizumab at a flat dose of 200 mg every 3 wk, or platinum-based chemotherapy for 4-6 cycles[17]. PD-L1 expression was assessed in formalin-fixed tumor specimens obtained at the time of diagnosis of the metastatic disease. Fine-needle aspirates were not considered appropriate. The primary endpoint of the study was PFS. A total of 1653 out of 1934 screened patients had evaluable PD-L1 material, and 500 (30.2%) patients had a PD-L1 positivity of 50% or greater. Median PFS was significantly longer in the pembrolizumab group [10.3 mo (95%CI: 6.7 to "not reached")] than in the chemotherapy group [6.0 mo (95%CI: 4.2-6.2)] with HR for disease progression or death of 0.50 (95%CI: 0.37-0.68, P < 0.001). The overall response rate was 44.8% (95%CI: 36.8%-53.0%) in the pembrolizumab group and 27.8% (95%CI: 20.8%-35.7%) in the chemotherapy group. At the time of the second interim analysis, OS was significantly longer with immunotherapy (HR for death: 0.60, 95%CI: 0.41-0.89, P = 0.005).

In the KEYNOTE-021 phase Ⅱ trial, a total of 123 treatment-naive advanced non-squamous NSCLC patients were randomized to receive 4 cycles of pembrolizumab (200 mg flat dose) plus carboplatin (AUC 5) and pemetrexed (500 mg/m^2) every 3 wk, followed by pemetrexed and pembrolizumab for 2 years, or to undergo the same strategy without pembrolizumab[18]. Randomisation was stratified by PDL-1 tumor proportion score (< 1% *vs* ⩾ 1%) assessed by the IHC 22C3 clone (Dako North America) in formalin-fixed tumour samples obtained at the time of diagnosis of metastatic disease. The primary end-point was the proportion of patients achieving an objective response. The response rate was 55% (95%CI: 42%-68%) in the pembrolizumab plus chemotherapy arm and 29% (95%CI: 18%-41%) in the standard arm with a 26% of difference in the response rate thus reaching statistical significance (95%CI: 9%-42%, P = 0.0016). In the experimental arm the response rate was 57% (95%CI: 34%-79%) in patients with a PDL-1 tumor score < 1% and 54% (95%CI: 37%-70%) in patients with a PDL-1 score of 1% or greater. Nevertheless, the probability of response increased according to the PD-L1 positivity level: 29% response rate in patients with PDL-1 positive tumors ranging from 1% to 49% and 80% response rate in those patients whose tumors scored 50% or greater (Table 2). Median PFS was longer with Pembrolizumab plus chemotherapy [13 mo (95%CI: 8.3 to "not reached")] with respect to chemotherapy alone [8.9 mo (95%CI: 4.4-10.3 mo)] with an HR of 0.53 (95%CI: 0.31-0.91, P = 0.01). However, no difference was observed in OS (HR = 0.90, 95%CI: 0.41-1.91, P = 0.39).

Anti- PD-L1 monoclonal antibodies

Atezolizumab

In the paper by Herbst and colleagues a total of 277 patients with advanced cancer were treated with escalating doses of MPDL3280A intravenously every 3 wk[19]. In advanced NSCLC patients (53/277 in total) the overall response rate was 21%. In this case PD-L1 was determined by using a novel IHC assay (Ventana SP142 North America) and positivity was categorized according to the expressing cell type [tumor cell (TC) or immune cell (IC)] and then scored along a gradient [< 1% (TC0 or IC0), 1%-4% (TC1 or IC1), 5%-49% (TC2) or 5%-10% (IC2), and ⩾ 50% (TC3) or <10% (IC3)]. A relationship was observed between PD-L1 scores and response rate: 83% of patients with score 3 responded to treatment, while only 20% of those with scores 0-2 obtained a remission (Table 3). However, not surprisingly, also 20% of patients with score 0 achieved a clinical response. In the subsequent randomized phase Ⅱ study (POPLAR) atezolizumab was compared to docetaxel, in terms of OS, in 285 pretreated advanced NSCLCs[20]. Patients were stratified according to the PD-L1 expression that was determined on TC as well as on IC by using the SP142 PD-L1 IHC assay (Ventana Medical Systems, Tucson, AZ, United States). The IHC scores were defined as follows: Score 0 =

Table 3 Correlation between atezolizumab activity and outcome and programmed cell death protein ligand 1 immunohistochemestry score

Author/study	Marker antibody	Tumor type	Treatment line	PD-L1 cutoff	N pts	Response (%)	mPFS mo (95%CI)	mOS mo (95%CI)
Atezolizumab							NR	NR
							NR	NR
							NR	NR
							NR	NR
Herbst *et al*[19]	Ventana SP142	NSCLC	≥ 2	Score 3	6	83	7.8 (2.7-12.3)	15.5 (9.8-NA)
Phase Ⅰ				Score 2	7	14	3.4 (1.4-6.9)	15.1 (8.4-NA)
				Score 1	13	15	3.0 (2.8-4.1)	15.5 (11.1-NA)
				Score 0	20	20	4.1 (2.7-5.6)	9.7 (6.7-12.0)
Fehrenbacher *et al*[20] POPLAR	Ventana SP142	NSCLC	≥ 2	Score 3	24	37.50	4.2 (2.9-7.0)	20.5 (17.5-NA)
Phase Ⅱ				Score 2	50	22.00	4.1 (2.8-5.3)	16.3 (13.3-20.1)
				Score 1	93	18.30	4.1 (2.9-4.3)	15.7 (12.6-18.0)
				Score 0	51	14.60	4.0 (3.1-4.2)	12.6 (9.6-15.2)
Rittmeyer *et al*[21]	Ventana	NSCLC	≥ 2	Score 3	72	30.60	7.3 (4.9-12.0)	26.9 (12.0-NA)
OAK	SP142			Score 2	129	22.50	7.3 (5.7-9.7)	23.5 (18.1-NA)
Phase Ⅲ				Score 1	241	17.80		
				Score 0	80	7.80	7.6 (4.0-9.7)	23.5 (18.1-NA)
Wakelee *et al*[22] and Antonia *et al*[23]	Ventana	NSCLC	1	Score 3	65	34		
BIRCH	SP142			TC2/3 or IC2/3	138	25		
Phase Ⅱ				Score 2	73	18		

Score 3: PDL1 expression levels TC3 or IC3 (≥ 50% on TC or ≥ 10% on IC); Score 2: TC2 or IC2 (≥ 5%-< 50% on TC or ≥ 5% -< 10% IC); Score 1: TC1 or IC1 (≥ 1% - < 5% on TC or IC); Score 0: TC0 and IC0 (< 1% on TC and IC). IC: Tumor-infiltrating immune cell; TC: Tumor cell; NR: Not reported; pts: Patients; NA: Not available; NSCLC: Non-small cell lung cancer; PD-L1: Programmed cell death protein ligand 1; mPFS: Median progression free survival; mOS: Median overall survival.

PD-L1 expression on IC or TC < 1%; score = 1 TC or IC PD-L1 positivity between ≥ 1 and < 5%; score = 2 positivity between ≥ 5 and < 50% on TC or PD-L1 expression on IC between ≥ 5 and < 10%; score = 3 PD-L1 positive TC ≥ 50% or PD-L1 positive IC ≥ 10%. Median OS in the atezolizumab arm was 12.6 mo (95%CI: 9.7-16.4) compared to 9.7 mo (95%CI: 8.6-12.0) in the docetaxel arm (HR = 0.73, 95%CI: 0.53-0.99; *P* = 0.04). Overall survival improves according to the PD-L1 score level: TC3 or IC3 HR 0.49, TC2/3 or IC2/3 HR 0.54, TC 1/2/3 or IC1/2/3 HR 0.59; TC0 or IC0 HR 1.04. PFS also varied according to the different PD-L1 subgroups, but the differences did not reach any statistical significance (Table 3). In the immunotherapy arm the overall response rate was 37.5%, 22.0%, 18.3% and 16.7% in TC3 or IC3, TC2/3 or IC2/3, TC 1/2/3 or IC1/2/3. In the subgroup TC0 or IC0, the response rates were similar (14.6%) in both arms (Table 3).

In the phase Ⅲ OAK trial, patients with squamous or non-squamous advanced NSCLC, pretreated with one or two chemotherapy regimens, were randomly assigned to 1200 mg of atezolizumab or 75 mg/m^2 of docetaxel every 3 wk[21]. The primary endpoint was OS. The mOS was 13.8 mo (95%CI: 11.8-15.7) in the atezolizumab arm and 9.6 mo (95%CI: 8.6-11.2) in the docetaxel arm (HR = 0.73, 95%CI: 0.62-0.87, *P* = 0.0003). Median OS was also analyzed according to the criteria of the previous study (20): In the TC1/2/3 or IC1/2/3 populations OS was 15.7 mo (95%CI: 12.6-18.0) with atezolizumab *vs* 10.3 (95%CI: 8.8-12.0) with docetaxel (HR = 0.74, 95%CI: 0.58-0.93, *P* = 0.0102) and in the TC0 or IC0 groups mOS was 12.6 mo *vs* 8.9 mo with atezolizumab and docetaxel respectively (HR = 0.75, 95%CI: 0.59-0.96). In the intention to treat population PFS did not differ between the two arms (HR = 0.95, 95%CI: 0.82-1.10, *P* = 0.4928) and in the different PD-L1 subgroups. Objective responses for atezolizumab were 30.6% in the TC3/IC3 subgroup, 22.5% in the TC 2/3 or IC 2/3 aubgroups, 17.8% and 7.8% in the TC 1/2/3 or IC1/2/3 and TC0 or IC0 subgroups, respectively (Table 3).

In phase Ⅱ Birch trial patients with advanced NSCLC received atezolizumab in first or subsequent line of treatment at a flat dose of 1200 mg every three weeks[22]. The PDL-1 expression was evaluated by using the Ventana SP142 IHC assay and the study enrolled only patients with PDL1 expression > 5% in tumor cells or in immune cells (TC2/3 or IC 2/3). Efficacy data in the first line setting have been reported in a recent update[23]. Patients with PDL-1 TC3 or IC3 showed a 34% response rate and a mOS of 26.9 mo; PDL-1 TC2/3 or IC2/3 scores had an overall response rate of 25% and a mOS of 23.5 mo, and patients with PDL-1 TC2 or IC2 scores had an overall response rate of 18% and a mOS of 23.5 mo (Table 3).

Durvalumab

An ongoing phase 1/2 study is evaluating the safety and efficacy of durvalumab in patients with advanced NSCLC or with other solid tumor types[23]. Durvalumab was administered at 10 mg/kg every two weeks in previously untreated advanced NSCLC. Fifteen patients were initially enrolled regardless of the PD-L1 status. After a protocol amendment, enrolment was restricted to PD-L1 positive patients. PD-L1 status was assessed

Table 4 Correlation between durvalumab and avelumab activity and outcome and programmed cell death protein ligand 1 immunohistochemestry score

Author/study	Marker antibody	Tumor type	Treatment line	PD-L1 cutoff	N pts	Response (%)	mPFS mo (95%CI)	mOS mo (95%CI)
Durvalumab								
Gulley *et al*[24]	Ventana SP263	NSCLC	1	⩾ 25%	43	25		
Phase 1/2				< 5%	8	12		
Avelumab								
Verschraegen *et al*[25]	?	NSCLC	⩾ 2	⩾ 1%	118	14.40	11.7 wk	NR
Phase 1b				< 1%	20	10	5.9 wk	NR
Sheng *et al*[26]	?	NSCLC	1	⩾ 1%	35	20	NR	NR
Javelin				< 1%	10	0	NR	NR
Phase Ib								

NR: Not reported; pts: Patients; NSCLC: Non-small cell lung cancer; PD-L1: Programmed cell death protein ligand 1; mPFS: Median progression free survival; mOS: Median overall survival.

with the companion Ventana SP263 assay. PD-L1 positivity was defined as a tumor cell membrane staining of ⩾ 25%. A total of 59 patients (48 PD-L1 positive; 9 PD-L1 negative) were included in the trial. The overall response rate was 25% in PDL-1 positive patients and 12% in PDL-1 negative patients (Table 4).

Avelumab

A phase-1b trial was designed to investigate the safety and activity of avelumab (MSB0010718C) in patients with advanced NSCLC progressing after platinum-based chemotherapy[24]. Patients were treated with avelumab at 10 mg/kg every two weeks. Tumor PD-L1 expression was assessed by immunohistochemistry. Objective responses were observed in 22 patients [12% (95%CI: 7.6%-17.5%)], while 70 patients (38%) achieved a stable disease. Median PFS was 11.6 wk (95%CI: 8.4-12.1). One hundred and eighteen (86%) evaluable patients were PDL1 positive (1% threshold of positivity). The overall response rate was 14.4% and 10.0% in PD-L1 positive and negative tumors, respectively. Median PFS in PD-L1 positive patients was 11.7 wk and 5.9 in PD-L1-negative patients.

The safety and activity of avelumab in chemotherapy-naive advanced NSCLC patients were investigated in a phase 1b trial[25]. Patients received 10 mg/kg of avelumab IV every 2 wk; PD-L1 expression was assessed by IHC with ⩾ 1% positivity threshold on tumor cell staining. The overall response rate was 18.7% (95%CI: 10.6, 29.3) and a disease stabilization was reported in 34 patients (45.3%). In 35 PD-L1 positive tumors the overall response rate was 20.0%; no patients with PD-L1 negative tumors achieved a response. Median PFS was 11.6 wk (95%CI: 6.7-17.9) for all treated patients (Table 4).

CONCLUSION

The literature data have clearly shown that immune checkpoint inhibitors might represent an important therapeutic option for NSCLC patients. However, in spite of exciting overall treatment outcomes, a considerable number of patients failed to achieve long-term clinical benefit.

Since the cost of these molecules impacts significantly on health care systems, the identification of predictive biomarkers to select patients who are more likely to benefit is a challenging area of ongoing research. The PD-L1 expression was early identified as potential indicator of benefit and the literature on this topic is plentiful. Several critical aspects might explain the conflicting results shown in clinical trials by using retrospective or prospective PD-L1 assays. Some of these results are strictly related to the PD-L1 nature, while others derive from the methodologies and material that have been used for testing. PD-L1 is a constitutively but also a functionally inducible receptor/ligand potentially expressed by tumor cells, stromal cells, inflammatory cells at tumor sites; it is heterogeneous and subject to pre-analytical variables. Furthermore, its expression is continuously distributed, it has varied significantly over time and may be affected by concurrent or prior treatments (radiation or chemotherapy)[26-28]. Classical predictive biomarkers such as hormone receptors, HER2 protein over-expression or gene amplification, EGFR activating mutations and ALK rearrangements are always present: These indicators define more clearly distinct tumor subgroups with different biology and clinical behavior. The PD-L1 expression is very dynamic, according to a constantly evolving immune response. Therefore, questions regarding reliability, consistency, feasibility and selection of an expression as a threshold remain artificial and controversial. This might explain why a significant proportion of PD-L1 negative patients benefited from treatment with immunotherapy in all studies. Conversely, even in highly PD-L1 selected cohorts, 25% to 50% of patients achieved no benefit. Moreover, it is not clear whether PD-L1 positivity has a different effect on outcome/response to treatment, compared to PD-L1 positivity on immune cells. PDL-1 expression was evaluated in tumor cells in the majority of studies. The immunoresponse is a delicate balance between inhibitory checkpoints and activating signals

such as LAG-3, OX40, *etc*. The discovery of these proteins has paved the way to new therapy strategies, whereas their potential predictive role as biomarkers of immunoresponse is actually unknown.

Technical aspects may also result in inconsistent data; tissue fixation, storage, and antigen recovery are not standardized. The quality of commercially available antibodies is also a reason for concern: The PD-L1 diagnostic test for nivolumab (Dako 28-8 pharmDx), pembrolizumab (Dako 22C3 pharmDx), atezolizumab (Ventana SP142) and durvalumab (Ventana SP263) showed variability in staining intensity and patterns creating uncertainties and doubts for their use in everyday practice. To address these concerns, som-eyears ago a task force was set up, formed by phar-maceutical companies, by representatives from Dako and Ventana, and by the scientific companies FDA, AACR, ASCO and IASCLC (International Association for the Study of Lung Cancer). The aim was to compare the performance of the four major PD-L1 companion assays. The recently published results of the pilot phase of the "Blueprint PDL1 IHC assay comparison project"[29] indicates that interchanging assays and cut-offs will lead to the misclassification of PD-L1 status for some patients, and therefore more data are required.

Summing up, the PD-L1 expression is likely to be related to the curative efficacy of immune checkpoint inhibitors. However, its role seems to be more informative in terms of probability and magnitude of the treatment effect rather than prediction of the effect itself, given that none of the available assays can conclusively identify non-benefitting patients.

REFERENCES

1 **Torre LA**, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. *CA Cancer J Clin* 2015; **65**: 87-108 [PMID: 25651787 DOI: 10.3322/caac.21262]

2 **Lee JK**, Hahn S, Kim DW, Suh KJ, Keam B, Kim TM, Lee SH, Heo DS. Epidermal growth factor receptor tyrosine kinase inhibitors vs conventional chemotherapy in non-small cell lung cancer harboring wild-type epidermal growth factor receptor: a meta-analysis. *JAMA* 2014; **311**: 1430-1437 [PMID: 24715074 DOI: 10.1001/jama.2014.3314]

3 **Shaw AT**, Kim DW, Nakagawa K, Seto T, Crinó L, Ahn MJ, De Pas T, Besse B, Solomon BJ, Blackhall F, Wu YL, Thomas M, O'Byrne KJ, Moro-Sibilot D, Camidge DR, Mok T, Hirsh V, Riely GJ, Iyer S, Tassell V, Polli A, Wilner KD, Jänne PA. Crizotinib versus chemotherapy in advanced ALK-positive lung cancer. *N Engl J Med* 2013; **368**: 2385-2394 [PMID: 23724913 DOI: 10.1056/NEJMoa1214886]

4 **Keir ME**, Butte MJ, Freeman GJ, Sharpe AH. PD-1 and its ligands in tolerance and immunity. *Annu Rev Immunol* 2008; **26**: 677-704 [PMID: 18173375 DOI: 10.1146/annurev.immunol.26.021607.090331]

5 **Avogadri F**, Yuan J, Yang A, Schaer D, Wolchok JD. Modulation of CTLA-4 and GITR for cancer immunotherapy. *Curr Top Microbiol Immunol* 2011; **344**: 211-244 [PMID: 20563707 DOI: 10.1007/82_2010_49]

6 **Chen DS**, Mellman I. Oncology meets immunology: the cancer-immunity cycle. *Immunity* 2013; **39**: 1-10 [PMID: 23890059 DOI: 10.1016/j.immuni.2013.07.012]

7 **Snyder A**, Makarov V, Merghoub T, Yuan J, Zaretsky JM, Desrichard A, Walsh LA, Postow MA, Wong P, Ho TS, Hollmann TJ, Bruggeman C, Kannan K, Li Y, Elipenahli C, Liu C, Harbison CT, Wang L, Ribas A, Wolchok JD, Chan TA. Genetic basis for clinical response to CTLA-4 blockade in melanoma. *N Engl J Med* 2014; **371**: 2189-2199 [PMID: 25409260 DOI: 10.1056/NEJMoa1406498]

8 **Gettinger SN**, Horn L, Gandhi L, Spigel DR, Antonia SJ, Rizvi NA, Powderly JD, Heist RS, Carvajal RD, Jackman DM, Sequist LV, Smith DC, Leming P, Carbone DP, Pinder-Schenck MC, Topalian SL, Hodi FS, Sosman JA, Sznol M, McDermott DF, Pardoll DM, Sankar V, Ahlers CM, Salvati M, Wigginton JM, Hellmann MD, Kollia GD, Gupta AK, Brahmer JR. Overall Survival and Long-Term Safety of Nivolumab (Anti-Programmed Death 1 Antibody, BMS-936558, ONO-4538) in Patients With Previously Treated Advanced Non-Small-Cell Lung Cancer. *J Clin Oncol* 2015; **33**: 2004-2012 [PMID: 25897158 DOI: 10.1200/JCO.2014.58.3708]

9 **Rizvi NA**, Mazières J, Planchard D, Stinchcombe TE, Dy GK, Antonia SJ, Horn L, Lena H, Minenza E, Mennecier B, Otterson GA, Campos LT, Gandara DR, Levy BP, Nair SG, Zalcman G, Wolf J, Souquet PJ, Baldini E, Cappuzzo F, Chouaid C, Dowlati A, Sanborn R, Lopez-Chavez A, Grohe C, Huber RM, Harbison CT, Baudelet C, Lestini BJ, Ramalingam SS. Activity and safety of nivolumab, an anti-PD-1 immune checkpoint inhibitor, for patients with advanced, refractory squamous non-small-cell lung cancer (CheckMate 063): a phase 2, single-arm trial. *Lancet Oncol* 2015; **16**: 257-265 [PMID: 25704439 DOI: 10.1016/S1470-2045(15)70054-9]

10 **Brahmer J**, Reckamp KL, Baas P, Crinò L, Eberhardt WE, Poddubskaya E, Antonia S, Pluzanski A, Vokes EE, Holgado E, Waterhouse D, Ready N, Gainor J, Arén Frontera O, Havel L, Steins M, Garassino MC, Aerts JG, Domine M, Paz-Ares L, Reck M, Baudelet C, Harbison CT, Lestini B, Spigel DR. Nivolumab versus Docetaxel in Advanced Squamous-Cell Non-Small-Cell Lung Cancer. *N Engl J Med* 2015; **373**: 123-135 [PMID: 26028407 DOI: 10.1056/NEJMoa1504627]

11 **Borghaei H**, Paz-Ares L, Horn L, Spigel DR, Steins M, Ready NE, Chow LQ, Vokes EE, Felip E, Holgado E, Barlesi F, Kohlhäufl M, Arrieta O, Burgio MA, Fayette J, Lena H, Poddubskaya E, Gerber DE, Gettinger SN, Rudin CM, Rizvi N, Crinò L, Blumenschein GR Jr, Antonia SJ, Dorange C, Harbison CT, Graf Finckenstein F, Brahmer JR. Nivolumab versus Docetaxel in Advanced Nonsquamous Non-Small-Cell Lung Cancer. *N Engl J Med* 2015; **373**: 1627-1639 [PMID: 26412456 DOI: 10.1056/NEJMoa1507643]

12 **Gettinger S**, Rizvi NA, Chow LQ, Borghaei H, Brahmer J, Ready N, Gerber DE, Shepherd FA, Antonia S, Goldman JW, Juergens RA, Laurie SA, Nathan FE, Shen Y, Harbison CT, Hellmann MD. Nivolumab Monotherapy for First-Line Treatment of Advanced Non-Small-Cell Lung Cancer. *J Clin Oncol* 2016; **34**: 2980-2987 [PMID: 27354485 DOI: 10.1200/JCO.2016.66.9929]

13 **Rizvi NA**, Hellmann MD, Brahmer JR, Juergens RA, Borghaei H, Gettinger S, Chow LQ, Gerber DE, Laurie SA, Goldman JW, Shepherd FA, Chen AC, Shen Y, Nathan FE, Harbison CT, Antonia S. Nivolumab in Combination With Platinum-Based Doublet Chemotherapy for First-Line Treatment of Advanced Non-Small-Cell Lung Cancer. *J Clin Oncol* 2016; **34**: 2969-2979 [PMID: 27354481 DOI: 10.1200/JCO.2016.66.9861]

14 **Socinski M**, Creelan B, Horn L, Reck M, Paz-Ares L, Steins M, Felip E, van den Heuvel M, Ciuleanu TE, Badin F, Ready N, Hiltermann TJN, Nair S, Juergens R, Peters S, Minenza E, Geese WJ, Bhagavatheeswaran P, Chen A, Carbone DP. NSCLC, metastatic CheckMate 026: A phase 3 trial of nivolumab vs investigator's choice (IC) of platinum-based doublet chemotherapy (PT-DC) as first-line therapy for stage iv/recurrent programmed death ligand 1 (PD-L1)-positive NSCLC. *Ann Oncol* 2016; **27**: LBA7_PR [DOI: 10.1093/annonc/mdw435.39]

15 **Garon EB**, Rizvi NA, Hui R, Leighl N, Balmanoukian AS, Eder JP, Patnaik A, Aggarwal C, Gubens M, Horn L, Carcereny E, Ahn MJ, Felip E, Lee JS, Hellmann MD, Hamid O, Goldman JW, Soria JC, Dolled-Filhart M, Rutledge RZ, Zhang J, Lunceford JK, Rangwala R, Lubiniecki GM, Roach C, Emancipator K, Gandhi L; KEYNOTE-001 Investigators. Pembrolizumab for the treatment of non-small-cell lung cancer. *N Engl J Med* 2015; **372**: 2018-2028 [PMID: 25891174 DOI: 10.1056/NEJMoa1501824]

16 **Herbst RS**, Baas P, Kim DW, Felip E, Pérez-Gracia JL, Han JY, Molina J, Kim JH, Arvis CD, Ahn MJ, Majem M, Fidler MJ, de Castro G Jr, Garrido M, Lubiniecki GM, Shentu Y, Im E, Dolled-

Filhart M, Garon EB. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. *Lancet* 2016; **387**: 1540-1550 [PMID: 26712084 DOI: 10.1016/S0140-6736(15)01281-7]

17 **Reck M**, Rodríguez-Abreu D, Robinson AG, Hui R, Csőszi T, Fülöp A, Gottfried M, Peled N, Tafreshi A, Cuffe S, O'Brien M, Rao S, Hotta K, Leiby MA, Lubiniecki GM, Shentu Y, Rangwala R, Brahmer JR; KEYNOTE-024 Investigators. Pembrolizumab versus Chemotherapy for PD-L1-Positive Non-Small-Cell Lung Cancer. *N Engl J Med* 2016; **375**: 1823-1833 [PMID: 27718847 DOI: 10.1056/NEJMoa1606774]

18 **Langer CJ**, Gadgeel SM, Borghaei H, Papadimitrakopoulou VA, Patnaik A, Powell SF, Gentzler RD, Martins RG, Stevenson JP, Jalal SI, Panwalkar A, Yang JC, Gubens M, Sequist LV, Awad MM, Fiore J, Ge Y, Raftopoulos H, Gandhi L; KEYNOTE-021 investigators. Carboplatin and pemetrexed with or without pembrolizumab for advanced, non-squamous non-small-cell lung cancer: a randomised, phase 2 cohort of the open-label KEYNOTE-021 study. *Lancet Oncol* 2016; **17**: 1497-1508 [PMID: 27745820 DOI: 10.1016/S1470-2045(16)30498-3]

19 **Herbst RS**, Soria JC, Kowanetz M, Fine GD, Hamid O, Gordon MS, Sosman JA, McDermott DF, Powderly JD, Gettinger SN, Kohrt HE, Horn L, Lawrence DP, Rost S, Leabman M, Xiao Y, Mokatrin A, Koeppen H, Hegde PS, Mellman I, Chen DS, Hodi FS. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. *Nature* 2014; **515**: 563-567 [PMID: 25428504 DOI: 10.1038/nature14011]

20 **Fehrenbacher L**, Spira A, Ballinger M, Kowanetz M, Vansteenkiste J, Mazieres J, Park K, Smith D, Artal-Cortes A, Lewanski C, Braiteh F, Waterkamp D, He P, Zou W, Chen DS, Yi J, Sandler A, Rittmeyer A; POPLAR Study Group. Atezolizumab versus docetaxel for patients with previously treated non-small-cell lung cancer (POPLAR): a multicentre, open-label, phase 2 randomised controlled trial. *Lancet* 2016; **387**: 1837-1846 [PMID: 26970723 DOI: 10.1016/S0140-6736(16)00587-0]

21 **Rittmeyer A**, Barlesi F, Waterkamp D, Park K, Ciardiello F, von Pawel J, Gadgeel SM, Hida T, Kowalski DM, Dols MC, Cortinovis DL, Leach J, Polikoff J, Barrios C, Kabbinavar F, Frontera OA, De Marinis F, Turna H, Lee JS, Ballinger M, Kowanetz M, He P, Chen DS, Sandler A, Gandara DR; OAK Study Group. Atezolizumab versus docetaxel in patients with previously treated non-small-cell lung cancer (OAK): a phase 3, open-label, multicentre randomised controlled trial. *Lancet* 2017; **389**: 255-265 [PMID: 27979383 DOI: 10.1016/S0140-6736(16)32517-X]

22 **Wakelee H**, Patel JD, Heist R, Balmanoukian A, Besse B, Felip E, Carcereny Costa E, Chow LQ, Koczywas M, Garassino MC, Christoph D, Toh CK, Johnson ML, Chaft J, Kurata T, Qiu J, Kowanetz M, Coleman S, Mocci S, Sandler A, Gettinger SN, Peters S. ORAL01.04: Phase II Trial of Atezolizumab for Patients with PD-L1-Selected Advanced NSCLC (BIRCH): Updated Efficacy and Exploratory Biomarker Results: Topic: Medical Oncology. *J Thorac Oncol* 2016; **11**: S251-S252 [PMID: 27969443 DOI: 10.1016/j.jtho.2016.09.009]

23 **Antonia SJ**, Kim S-W, Spira AI, Ahn M-J, Ou S-HI, Stjepanovic N, Fasolo A, Jäger D, Ott PA, Wainberg ZA, Wakelee HA, Goldman JW, Kurland J, Rebelatto MC, Yao W, Gupta AK, Blake-Haskins JA, Howard Segal N. Safety and clinical activity of durvalumab (MEDI4736), an anti-PD-L1 antibody, in treatment-naïve patients with advanced non−small-cell lung cancer. *J Clin Oncol* 2016; **34**: 9029 [DOI: 10.1200/JCO.2016.34.15_suppl.9029]

24 **Gulley JL**, Spigel D, Kelly K, Chandler JC, Rajan A, Hassan R, Wong DJL, Leach J, Edenfield WJ, Wang D, Vrindavanam N, Weiss GJ, Gurtler JS, Grote HJ, von Heydebreck A, Chin KM, Iannotti N. Avelumab (MSB0010718C), an anti-PD-L1 antibody, in advanced NSCLC patients: A phase 1b, open-label expansion trial in patients progressing after platinum-based chemotherapy. *J Clin Oncol* 2015; **33**: 8034 [DOI: 10.1200/jco.2015.33.15_suppl.8034]

25 **Verschraegen CF**, Chen F, Spigel DR, Iannotti N, McClay EF, Redfern CH, Bennouna J, Taylor MH, Kaufman H, Kelly K, Bajars M, von Heydebreck A, Cuillerot JM, Jerusalem GHM. Avelumab (MSB0010718C; anti-PD-L1) as a first-line treatment for patients with advanced NSCLC from the JAVELIN Solid Tumor phase 1b trial: Safety, clinical activity, and PD-L1 expression. *J Clin Oncol* 2016; **34**: 9036 [DOI: 10.1200/JCO.2016.34.15_suppl.9036]

26 **Sheng J**, Fang W, Yu J, Chen N, Zhan J, Ma Y, Yang Y, Huang Y, Zhao H, Zhang L. Expression of programmed death ligand-1 on tumor cells varies pre and post chemotherapy in non-small cell lung cancer. *Sci Rep* 2016; **6**: 20090 [PMID: 26822379 DOI: 10.1038/srep20090]

27 **Deng L**, Liang H, Burnette B, Beckett M, Darga T, Weichselbaum RR, Fu YX. Irradiation and anti-PD-L1 treatment synergistically promote antitumor immunity in mice. *J Clin Invest* 2014; **124**: 687-695 [PMID: 24382348 DOI: 10.1172/JCI67313]

28 **Twyman-Saint Victor C**, Rech AJ, Maity A, Rengan R, Pauken KE, Stelekati E, Benci JL, Xu B, Dada H, Odorizzi PM, Herati RS, Mansfield KD, Patsch D, Amaravadi RK, Schuchter LM, Ishwaran H, Mick R, Pryma DA, Xu X, Feldman MD, Gangadhar TC, Hahn SM, Wherry EJ, Vonderheide RH, Minn AJ. Radiation and dual checkpoint blockade activate non-redundant immune mechanisms in cancer. *Nature* 2015; **520**: 373-377 [PMID: 25754329 DOI: 10.1038/nature14292]

29 **Hirsch FR**, McElhinny A, Stanforth D, Ranger-Moore J, Jansson M, Kulangara K, Richardson W, Towne P, Hanks D, Vennapusa B, Mistry A, Kalamegham R, Averbuch S, Novotny J, Rubin E, Emancipator K, McCaffery I, Williams JA, Walker J, Longshore J, Tsao MS, Kerr KM. PD-L1 Immunohistochemistry Assays for Lung Cancer: Results from Phase 1 of the Blueprint PD-L1 IHC Assay Comparison Project. *J Thorac Oncol* 2017; **12**: 208-222 [PMID: 27913228 DOI: 10.1016/j.jtho.2016.11.2228]

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