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ORIGINAL ARTICLE

PD-L1 protein expression assessed by immunohistochemistry is neither prognostic nor predictive of benefit from adjuvant chemotherapy in resected non-small cell lung cancer

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Background: The expression of programmed death (PD) ligand 1 (PD-L1) protein expression assessed by immunohistochemistry (IHC) has been correlated with response and survival benefit from anti-PD-1/PD-L1 immune checkpoint inhibitor therapies in advanced non-small cell lung carcinoma (NSCLC). The efficacy of several agents appears correlated with PD-L1 expression. It remains controversial whether PD-L1 is prognostic in NSCLC. We assessed the prognostic value of PD-L1 IHC and its predictive role for adjuvant chemotherapy in early stage NSCLC.

Patients and methods: Tumor sections from three pivotal adjuvant chemotherapy trials (IALT, JBR.10, CALGB 9633) using the E1L3N antibody were studied in this pooled analysis. PD-L1 staining intensity and percentage in both tumor cells (TCs) and immune cells (ICs) were scored by two pathologists. The average or consensus PD-L1 expression levels across intensities and/or percent cells stained were correlated with clinicopathological and molecular features, patient survivals and potential benefit of adjuvant chemotherapy.

Results: Results from 982 patients were available for analysis. Considering staining at any intensities for overall PD-L1 expression, 314 (32.0%), 204 (20.8%) and 141 (14.3%) tumor samples were positive for PD-L1 staining on TCs using cut-offs at $\geq$1%, $\geq$10% and $\geq$25%, respectively. For PD-L1 expressing ICs, 380 (38.7%), 308 (31.4%) and 148 (15.1%) were positive at $\geq$ 1%, $\geq$10% and 25% cut-offs, respectively. Positive PD-L1 was correlated with squamous histology, intense lymphocytic infiltrate, and *KRAS* but not with *TP53* mutation. *EGFR* mutated tumors showed statistically non-significant lower PD-L1 expression. PD-L1 expression was neither prognostic with these cut-offs nor other exploratory cut-offs, nor were predictive for survival benefit from adjuvant chemotherapy.

Conclusions: PD-L1 IHC is not a prognostic factor in early stage NSCLC patients. It is also not predictive for adjuvant chemotherapy benefit in these patients.

Key words: immunotherapy, PD-L1, immune cells, checkpoint inhibitors, E1L3N antibody, LACE-Bio

Introduction

Anticancer immunotherapy targeting immune checkpoints with antibodies to programmed death-1 (PD-1) and its ligand PD-L1 is an established treatment modality for non-small cell lung cancer (NSCLC) [1–6]. PD-L1 protein expression assessed by immuno-chemistry (IHC) has emerged as a biomarker to select NSCLC patients for pembrolizumab therapy [2, 4, 5]. Data from the second-line phase 3 trials of nivolumab and atezolizumab in NSCLC showed increasingly greater efficacy compared with chemotherapy in patients whose tumors expressed PD-L1 on tumor cells (TCs) and/or immune cells (ICs) [3, 6]. Recently, first-line pembrolizumab has demonstrated significantly longer survival compared with chemotherapy for PD-L1 IHC positive NSCLC [5]. Trials of some of these agents have moved into the adjuvant setting for early stage resected NSCLC patients. In this setting, it will be important to know whether PD-L1 expression is a prognostic marker for these patients. To date, several institutional series assessing the latter have been reported, but the results have been inconsistent (Table 1). Furthermore, as adjuvant chemotherapy has become standard of care in stage II–IIIA patients, the potential impact of PD-L1 expression on adjuvant chemotherapy in operable lung cancer patients is also important to determine. There are different ways by which platinum may induce immunogenic cell death providing rational for immune checkpoint combinations with cisplatin therapy [7–9]. Recently, the phase 2 KEYNOTE 021 trial reported a potential enhancing effect of pembrolizumab with chemotherapy in first-line advanced NSCLC [10].

The Lung Adjuvant Cisplatin Evaluation Biomarker (LACE-Bio) collaborative group has conducted several pooled analyses or validation studies on promising biomarkers in a large cohort of patients who participated in four pivotal adjuvant chemotherapy trials: IALT [11], ANITA, JBR.10, and CALGB 9633. These prospectively randomized controlled phase 3 trials provided a unique opportunity to study both the prognostic and predictive value of PD-L1 in early stage NSCLC, in light of many biological relations of immune checkpoint PD-L1/PD1 and chemotherapy. This study also provided the opportunity to gain insight into potential relationships of PD-L1 expression with several immune-related factors that previously have been investigated in the LACE-Bio patients, including tumor histology [11], lymphocytic infiltration [12], and mutation status of *EGFR* [13], *KRAS* [14], and *TP53* [15].

Patients and methods

Patients and pathology materials

This study included only LACE-Bio patients from the IALT, JBR.10, and CALGB 9633 trials. Among 1608 patients in this cohort, 1008 patients had one representative formalin-fixed paraffin embedded tumor block available for assessment. Attrition due to lack of adequate tumor tissue in the block or technical failure, resulted in 982 patients with evaluable PD-L1 stained sections (supplementary Figure S1, available at *Annals of Oncology* online).

PD-L1 immunohistochemistry and scoring

PD-L1 immunohistochemistry (IHC) was carried out on 4 µm sections, using the E1L3N rabbit monoclonal antibody (Cell Signaling, Danvers,

MA) on BenchMark XT autostainer (Ventana Medical Systems, Tucson, AZ). The details of staining protocols are described in Supplementary (S) Materials. Slides were assessed independently by the two study pathologists (EB, MST) for percent TCs showing membranous PD-L1 staining (TC) and for percent area of tumor infiltrating ICs showing PD-L1 staining (IC), at any intensity. IALT cases were screened by EB, and JBR.10/CALGB cases were screened by MST. Cases that showed TC or IC PD-L1 stained cells were identified for cross evaluation by the second pathologist (EB: JBR.10/CALGB; MT: IALT). When the scores of the two readers showed >20% difference, the slides were re-assessed independently by both pathologists. The final scores were the average of two closest scores by the two pathologists. The scoring was carried out blinded to clinical data/endpoints.

Statistical analysis

Overall survival (OS), the main endpoint, was defined as the time from randomization to death from any cause and disease-free survival (DFS) as the time from randomization to disease recurrence or death from any cause, whichever came first. The association of clinico-pathological variables and tumor and IC PD-L1 staining was studied using logistic regression stratified by trial. The prognostic value was estimated in the observation arm and its heterogeneity across histology and trial investigated by interaction terms. The predictive value was also estimated by adding an interaction term between treatment and PD-L1 and its heterogeneity across trials investigated. To be consistent with the PD-L1 cut-offs evaluated/adopted in various anti-PD1/PD-L1 therapeutic trials [2–6, 20] TC (1%, 25%, and 50%) and IC (1%, 10%, and 25%) were evaluated. *P*-values were two-sided and alpha level set to 5% for correlation analyses and 1% for prognostic and predictive analyses (pooled analysis). Additional details are provided in Supplementary Materials.

Results

Altogether 982 NSCLC patients had PD-L1 staining results available for the current analyses (supplementary Table S1, available at *Annals of Oncology* online). For these patients, the median follow-up was 5.3 years [range: 0.25–11.28] with 457 (46.5%) deaths and 520 (53.0%) events. The cohort had more males (72.6%) and T2 (76.1%), N0 (51.0%) patients, but had comparable proportions of squamous carcinoma and adenocarcinoma. However, 14% of patients were classified as other (not squamous or adenocarcinoma) NSCLC. *EGFR* mutation was found in 35 of the 300 (11.7%) adenocarcinoma patients successfully assayed, while *KRAS* mutation prevalence was at 33.0% (127/385) in adenocarcinoma. The characteristics of these 982 patients did not differ from 626 patients excluded because of lack of tissue or unsuccessful PD-L1 assessment, except for WHO PS ($P \leq 0.001$), T-stage ($P \leq 0.001$), and N-stage ($P = 0.01$) (supplementary Table S2, available at *Annals of Oncology* online). Excluded patients had more N0 tumors, WHO PS = 0.

PD-L1 IHC

Among the 982 patients, 314 (32.0%), 204 (20.8%), and 141 (14.3%) had TC of $\geq$1%, $\geq$25%, and $\geq$50%, respectively. For IC, 380 (38.7%), 308 (31.4%), and 148 (15.1%) had $\geq$1%, $\geq$10%, and $\geq$25%, respectively (supplementary Table S3, available at *Annals of Oncology* online).

Association of PD-L1 expression and clinical pathological factors

PD-L1 expression for TC and IC were not significantly associated in both univariate and multivariable analyses with sex, age, WHO

Table 1. Published reports that have studied the prognostic value of PD-L1 expression in non-small cell lung cancer or its subtypes

Author (year)	Ethnicity (country)	PD-L1 Ab	Ab nature (epitope)	Patient number	Sample type	Tumor histology	Tumor stage	Method	Staining feature	Cut-off	PD-L1 +/high patient (%)	Prognostic (OS) for +/High PD-L1 patients	Correlated with PD-L1 expression
Velcheti (2014)[c]	Caucasian (USA)	5H1 (JHU)	Mouse MC (extra-cellular)	155	TMA	NSCLC	I–IV	QIF/AQUA	Tumor cell membrane	>normal lung	36.1	Good	Tumor infiltrating lymphocytes (TIL)
	Caucasian (Greece)			303			I–IV				24.8	Good	
Schalper (2015)	Caucasian (USA)	E1L3N (CST)	Rabbit PC (intra-cellular)	202	TMA	NSCLC	I–IV	QIF/AQUA	Tumor cell membrane	Not specified	16.9	No	CD8+ TIL
	Caucasian (Greece)			350			I–IV				20.0	No	CD8+ TIL
Tang (2015)	East Asian (China)	E1L3N (CST)		170	Full section	NSCLC	IIB-IV	IHC	Membrane	H-score $\geq$5%	65.9	No	None with EGFR
Kim (2015)	East Asian (Korean)	E1L3N (CST)		331	TMA	SqCC	I–III	IHC	Membrane	$\geq$10% (any intensity)	26.9	No	CD8+ TIL
Schmidt (2015)	Caucasian (German)	E1L3N (CST)		321	TMA	NSCLC	I–III	IHC	Membrane	$\geq$5% 2 + (moderate)	24	No	None
Amera-tunga (2016)	Caucasian (Australia)	E1L3N (CST)		420	TMA	NSCLC	I–III	IHC	Membrane	$\geq$5% 2 + (moderate) $\geq$50% 2 + (moderate)	43.6 23.8	No (AdC and SqCC) Poor in N1 Good in N2	Strong PD-L1 ($\geq$50%): None with KRAS, EGFR Yes with FOXP3+ EGFR
Cooper (2015)	Caucasian (Australian)	22C3 (Merck)	Mouse MC (extra-cellular)	678	TMA	NSCLC	I–III	IHC	Membrane	$\geq$50% (any intensity)	7.7	Good	Not with ALK, EGFR and KRAS
Sun (2016)	East Asian (Korea)	22C3 (Merck)		1070	Full section	NSCLC	I–IV	IHC	Membrane	$\geq$1% (any intensity) $\geq$50% (any intensity)	45 6	No Borderline worse ($P = 0.05$)	Not analyzed
Chen (2012)	East Asian (China)	236A/E7 (abcam)	FOXP3 Ab (unknown)	120	Full section	NSCLC	I–III	IHC	Membrane and/or cytoplasm	IRS[a] $\geq$3	57.5	Poor	None reported
Zhang (2014)	East Asian (China)	SAB 2900365 (Sigma-Aldrich)	Rabbit PC (unknown)	143	Full section	AdC	I–III	IHC	Membrane and cytoplasm	'Quickscore' $\geq$8 (range 0–16)	49	Poor	Not with EGFR,KRAS, ALK
Yang (2014)	East Asian (Taiwan)	Proteintech	Rabbit PC (unknown)	163	Full section	AdC	I	IHC	Membrane	$\geq$5% (any intensity)	39.9	None	None with EGFR,KRAS, ALK, BRAF
Azuma (2014)	East Asian (Japan)	Lifespan Bioscience	Rabbit PC (unknown)	164	Full section	NSCLC	I–III	IHC	Membrane and cytoplasm	H-score >30% (median)	50	Poor	Yes with never smokers, AdC and EGFR mutant
Lin (2015)	East Asian (China)	ab58810 (Abcam)	Mouse MC (Center of CD274)	63	Biopsy	EGFR mutant AdC[b]	Advanced	IHC	Membrane and cytoplasm	Mean IRS score[a]	53.6	Good	Not with CD4/CD8 TIL

AdC, adenocarcinoma; JHU, Johns Hopkin University; CST, cell signaling technology; TMA, tissue microarray; NSCLC, non-small cell lung carcinoma; MC, monoclonal; PC, polyclonal; SqCC, squamous cell carcinoma.

[a]IRS, intensity (0–3) × percentage stained cells: 0, negative; 1: 1%–10%, 2: 11%–50%, 3: 51%–80%, 4: ≥80%. 3: +1 in >50% cells, or 2+ in >10% cells, or 3+ in ≥1% of cells.

[b]EGFR TKI treated.

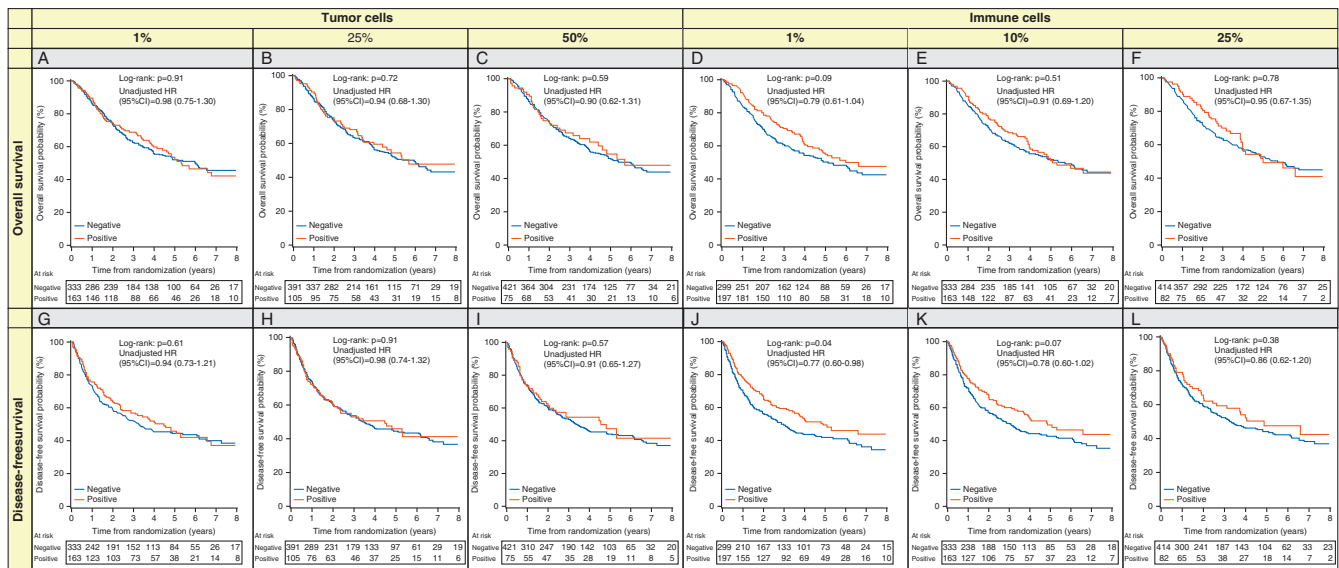[c]Full references are provided in Supplementary (S) Materials.

Figure 1. Kaplan–Meier plots showing lack of prognostic value of PD-L1 staining on tumor cell (TC) or immune cell (IC), using different pre-specified cut-offs (25% and 1%), in the observation arm patients ($n = 496$) with positive vs. negative PD-L1 staining. (A–D) overall survival; (E–H) disease-free survival.

PS, type of surgery, T and N of TNM, and stage, except for univariate N stage for IC 1% (supplementary Table S4, available at *Annals of Oncology* online). PD-L1 TC expression at all three cut-offs was significantly more prevalent in squamous than adenocarcinoma (univariate analysis). Similarly, significantly higher expression in squamous carcinoma also was observed for IC infiltrate at 1% and 10% cut-offs. Higher TC expression was noted in poorly differentiated NSCLC including large cell/adenosquamous/pleomorphic/sarcomatoid carcinomas (supplementary Table S4, available at *Annals of Oncology* online), but lower expression in large cell neuroendocrine carcinoma. Among adenocarcinoma subtypes, solid predominant tumors demonstrated significantly higher PD-L1 TC staining than other adenocarcinoma subtypes, for TC cut-offs 1%, 25%, and 50%, and IC at 1% and 10% (supplementary Table S5, available at *Annals of Oncology* online). Marked or intense tumor lymphocytic infiltration correlated marginally with greater frequency of PD-L1 expression on TC only in univariate analysis and significantly on IC in a multivariable analyses.

Association of PD-L1 expression and EGFR, KRAS and TP53 mutation

TC and IC PD-L1 staining was lower in *EGFR* mutant compared with wild-type (WT) adenocarcinoma, although the differences were not significant (supplementary Table S4, available at *Annals of Oncology* online). Conversely, *KRAS* mutant tumors showed significantly higher frequency of tumor PD-L1 expression (1% and 25%) compared with WT *KRAS* in multivariable analyses (see footnote of supplementary Table S4, available at *Annals of Oncology* online). PD-L1 staining was not significantly different between *TP53* mutant and WT NSCLC.

Prognostic analyses of PD-L1 IHC

At 1% cut-off, the unadjusted OS curves in the observation arm (Figure 1A) showed no significant difference (TC PD-L1 negative vs positive log rank $P = 0.91$). Similar results were observed for DFS (Figure 1G; log rank $P = 0.61$). A marginal effect was found with IC for both OS (HR = 0.79, 95% CI 0.69–1.04, $P = 0.09$) and DFS (HR = 0.77, 95% CI 0.60–0.98, $P = 0.04$) (Figure 1D and J). Multivariable analyses confirmed the lack of significant prognostic value (Table 2). The proportional hazards assumption was not violated (data not shown). These results were homogeneous across histologies (supplementary Table S6, available at *Annals of Oncology* online) and trials (supplementary Table S7, available at *Annals of Oncology* online). With 10%, 25%, or 50% cut-off, no prognostic effect was observed either for TC (Figure 1B, C, H, and I) or IC (Figure 1E, F, K, and L), for OS (Figure 1B, C, E, and F) and DFS (Figure 1H, I, K, and L), respectively. Multivariable analyses confirmed the lack of prognostic value (Table 2) with a marginal heterogeneity across trials for OS and IC (1%) ($P = 0.04$) (supplementary Table S7, available at *Annals of Oncology* online).

Predictive analyses for adjuvant chemotherapy benefit

At 1% cut-off, interaction P-value TC was 0.78 and 0.83 for OS and DFS, respectively and for IC, was 0.13 and 0.12 for OS and DFS, respectively (Table 3; Figure 2A, B, G, and H). However, we observed marginal heterogeneity across trials for OS and IC (1%) ($P = 0.06$) (supplementary Table S8, available at *Annals of Oncology* online). For TC ≥25% and ≥50%, the OS multivariable hazard ratio (HR) for post-operative chemotherapy compared with observation for positive was 0.90 (95% CI 0.59–1.37) and 0.78 (95% CI 0.46–1.32), while the HR for negative was 1.00 (95% CI 0.81–1.23) and 1.01 (95%CI 0.83 = 1.24), respectively (Table 3; Figure 2C, D, E, and F). The interaction P-value was not significant ($P = 0.67$ and 0.37). Similar results were obtained for DFS (P-interaction 0.33 and 0.18, respectively) (Table 3; Figure 2I, J, K, and L). These results were homogeneous across trials (supplementary Table S8, available at *Annals of Oncology* online).

Cut-offs	Overall survival			Disease-free survival		
	No. deaths/ no. patients	HR for death [95% CI]	P-value	No. events/ no. patients	HR for event [95% CI]	P-value
Tumor cells (N = 478)						
1%						
Negative	151/319	1.00	0.93	179/319	1.00	0.82
Positive	73/159	1.01 [0.76–1.35]		85/159	0.97 [0.74–1.27]	
25%						
Negative	178/375	1.00	0.96	208/375	1.00	0.62
Positive	46/103	0.99 [0.71–1.39]		56/103	1.08 [0.80–1.47]	
50%						
Negative	193/405	1.00	0.86	226/405	1.00	0.98
Positive	31/73	0.96 [0.65–1.43]		38/73	1.00 [0.70–1.44]	
Immune cells (N = 478)						
1%						
Negative	144/286	1.00	0.10	169/286	1.00	0.10
Positive	80/192	0.79 [0.59–1.05]		95/192	0.80 [0.62–1.05]	
10%						
Negative	155/320	1.00	0.50	187/320	1.00	0.16
Positive	69/158	0.90 [0.67–1.22]		77/158	0.82 [0.62–1.08]	
25%						
Negative	187/400	1.00	0.97	224/400	1.00	0.54
Positive	37/78	0.99 [0.69–1.44]		40/78	0.90 [0.63–1.27]	

Table 2. Prognostic values of PD-L1-based markers (tumor and immune cells) estimated from a multivariable Cox regression model stratified by trial in the observation arm (n = 478)[a]

HR, hazard ratio; CI, confidence interval.
[a]Eighteen observations were excluded because of missing values for covariates.

No differences in HRs were observed for IC at 10% and 25% cut-offs, both for OS and DFS with interaction P-values ranging from 0.25 to 0.94 (Table 3; supplementary Figure S2C–F and I–L, available at *Annals of Oncology* online).

Discussion

In this large and unique cohort of PD-L1 assessment in early stage resected NSCLC, we have demonstrated that PD-L1 expression in tumor and ICs is neither prognostic nor predictive for survival benefit from adjuvant platinum-based chemotherapy, using 1%, 25%, and 50% cut-offs for TC, and 1%, 10%, and 25% for IC staining. We also found that in multivariable correlation analyses, more frequent TC PD-L1 staining (1% and 25% cut-offs) was significantly associated with non-adenocarcinoma NSCLC and *KRAS* mutation, but not with *EGFR* or *TP53* mutation; the latter 2 markers might be affected by the high number of missing data. While *EGFR* mutated tumors appear to show lower PD-L1 expression, the difference did not reach statistical significance. Greater IC PD-L1 expression was significantly associated only with intense lymphocytic infiltrate.

Many PD-L1 antibodies are available to study PD-L1 expression in tumors, but not all antibodies have been well characterized for their specificity. McLaughlin et al. [16] reported that among 9 antibodies (5H1, E1L3N, E1J2J, Abcam 58810, 29E.2A3, MIH1, 27A2, GTX89590, 015) tested for staining specificities, only three (5HI, E1L3N and E1J2J) were specific for PD-L1.

However, four other antibodies (22C3, 28-8, SP142, and SP263) have been developed independently by different pharmaceutical/diagnostic companies as PD-L1 biomarker for their respective anti-PD1/PD-L1 agents [17]. Until recently, these latter antibodies except SP142 (Ventana, Tucson, AZ) were not available commercially for academic studies and evaluation. However, recently, Rimm et al. [18] compared the staining performance of E1L3N with 28-8 and 22C3 and found them to be comparable. We used the E1L3N antibody in our study, as when our study was initiated, the 22C3, 28-8, and SP-263 clones were not commercially available.

As anti-PD1 clinical trials are adopting their cut-offs regardless of staining intensity [3, 4], we focused our analyses using this approach and selecting 1% (second line nivolumab, pembrolizumab), 25% (proposed for durvalumab), and 50% (first line pembrolizumab) as cut-offs. With these cut-offs, TC was 32%, 20.8%, and 14.4% PD-L1 positive, respectively. While these rates were lower than those reported in advanced NSCLC from phase 3 nivolumab (CheckMate 017 and 057) [1, 3] and pembrolizumab (KEYNOTE 10) trials [4], they were within range of positivity rates reported in other studies that evaluated the prognostic value of PD-L1 staining in early stage NSCLC (Table 1; supplementary Table S9, available at *Annals of Oncology* online). Nevertheless, proper comparison of PD-L1 expression in advanced vs. early stage NSCLC tumors is warranted.

Using the 1%, 25%, and 50% cut-offs for TC and 1%, 10%, and 25% for IC, PD-L1 expression was not prognostic in the LACE-Bio patients who received surgery alone. Exploratory analyses were carried out to study the prognostic value of PD-L1 using different

Table 3. Predictive values of PD-L1-based markers (tumor and immune cells) estimated from a multivariable Cox regression model stratified by trial (n = 947)[a]

	Overall survival			Disease-free survival		
	Chemotherapy	Observation	HR for death CT vs. no CT [95% CI]	Chemotherapy	Observation	HR for event CT vs. no CT [95% CI]
	No. deaths/No. patients			No. events/No. patients		
Tumor cells (N = 947)						
1%						
Negative	147/319	151/319	0.96 [0.76–1.21]	163/319	179/319	0.86 [0.69–1.07]
Positive	70/150	73/159	1.02 [0.73–1.41]	74/150	85/159	0.90 [0.65–1.23]
HR for event Pos vs. neg [95% CI]	1.08 [0.81–1.45]	1.02 [0.77–1.36]	Interaction: P = 0.78	1.03 [0.78–1.35]	0.99 [0.76–1.28]	Interaction: P = 0.83
25%						
Negative	173/371	178/375	1.00 [0.81–1.23]	190/371	208/375	0.91 [0.75–1.11]
Positive	44/98	46/103	0.90 [0.59–1.37]	47/98	56/103	0.73 [0.49–1.08]
HR for event Pos vs. neg [95% CI]	0.89 [0.64–1.25]	0.99 [0.71–1.37]	Interaction: P = 0.67	0.88 [0.64–1.22]	1.10 [0.81–1.48]	Interaction: P = 0.33
50%						
Negative	190/403	193/405	1.01 [0.83–1.24]	209/403	226/405	0.92 [0.76–1.11]
Positive	27/66	31/73	0.78 [0.46–1.32]	28/66	38/73	0.64 [0.39–1.05]
HR for event Pos vs. neg [95% CI]	0.76 [0.50–1.14]	0.98 [0.66–1.45]	Interaction: P = 0.37	0.72 [0.48–1.07]	1.04 [0.73–1.47]	Interaction: P = 0.18
Immune cells (N = 947)						
1%						
Negative	132/288	144/286	0.87 [0.68–1.10]	145/288	169/286	0.78 [0.62–0.97]
Positive	85/181	80/192	1.17 [0.86–1.60]	92/181	95/192	1.04 [0.78–1.39]
HR for event Pos vs. neg [95% CI]	1.06 [0.80–1.41]	0.79 [0.59–1.04]	Interaction: P = 0.13	1.06 [0.81–1.39]	0.80 [0.62–1.03]	Interaction: P = 0.12
10%						
Negative	152/325	155/320	0.94 [0.75–1.18]	166/325	187/320	0.81 [0.66–1.00]
Positive	65/144	69/158	1.06 [0.75–1.49]	71/144	77/158	1.02 [0.74–1.41]
HR for event Pos vs. neg [95% CI]	1.03 [0.77–1.39]	0.92 [0.68–1.23]	Interaction: P = 0.57	1.01 [0.76–1.35]	0.81 [0.61–1.06]	Interaction: P = 0.25
25%						
Negative	190/403	187/400	0.98 [0.80–1.20]	208/403	224/400	0.86 [0.71–1.04]
Positive	27/66	37/78	0.96 [0.58–1.59]	29/66	40/78	0.90 [0.55–1.46]
HR for event Pos vs. neg [95% CI]	0.96 [0.64–1.44]	0.98 [0.68–1.40]	Interaction: P = 0.94	0.90 [0.60–1.33]	0.86 [0.61–1.21]	Interaction: P = 0.87

HR, hazard ratio; CI, confidence interval; CT, chemotherapy; no CT, observation; neg, negative (defined as < cut-off); pos, positive (defined as ≥ cut-off).
[a]Thirty-five observations were excluded because of missing values for covariates.

approaches (design variables formed from the quartiles, martingale residuals and data-oriented approach). In fact, exploratory analyses with 10% increments, as well as design variables formed from the quartiles, martingale residuals and data-oriented approach for studying the form of continuous markers (non-linear association), did not identify any cut-off that made PDL1 a prognostic factor (supplementary Figure S3, available at *Annals of Oncology* online). As additional prognostic analyses, we considered the proposed adenocarcinoma grading in 3 groups (low, intermediate and high grade).[19] While no patient was classified into low grade, 174 (17.7%) and 215 (21.9%) patients were classified in intermediate and high grade, respectively. Consistent with our previous report [11], we observed a significant overall prognostic value of histology (squamous cell carcinoma, adenocarcinoma intermediate grade, adenocarcinoma high grade, and other

NSCLC) for DFS but not for OS, with high grade adenocarcinoma patients having significantly poorer prognosis. However, the prognostic value of PD-L1-based markers remains not significant when considering histology with adenocarcinoma histological subtypes (data not shown).

This study presented a unique opportunity to evaluate the potential role of PD-L1 expression in the benefit of adjuvant chemotherapy, a standard of care for stage II-IIIA NSCLC. We have shown that PD-L1 expression has no differential effect on the survival benefit from adjuvant chemotherapy in early stage completely resected NSCLC patients (Table 3). This information may be useful in the future when interpreting the results of immune checkpoint therapies in the adjuvant setting in the BR.31 [NCT02273375], KEYNOTE-091/PEARLS [NCT02504372], and ANVIL [NCT02595944] trials.
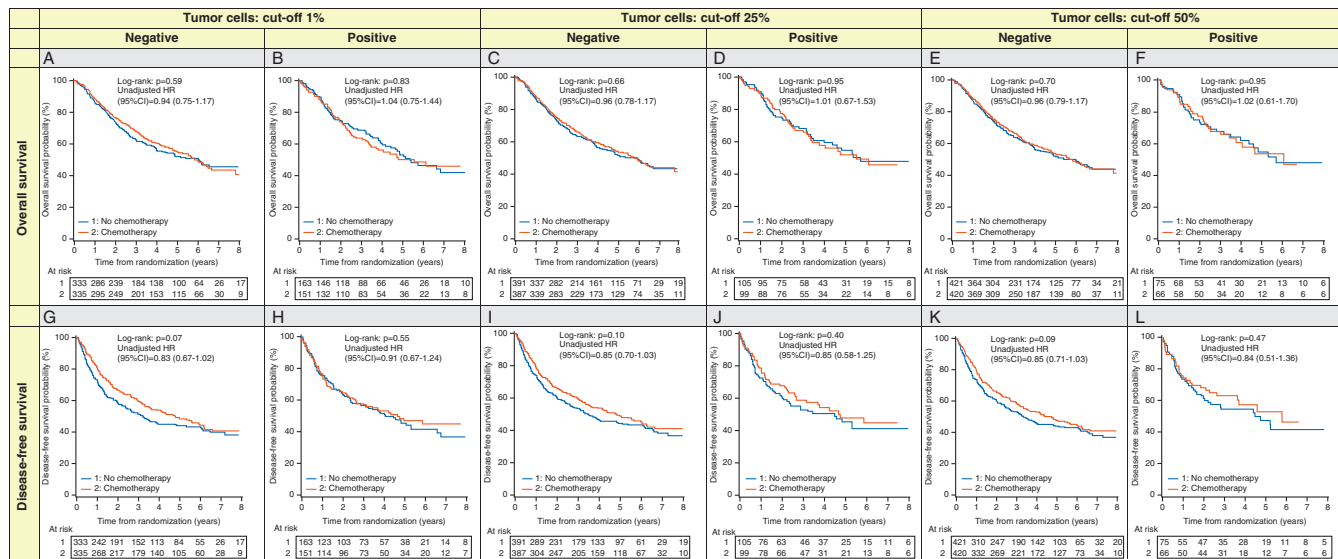
Figure 2. Kaplan–Meier plots showing lack of predictive value of PD-L1 staining on tumor cell, between chemotherapy and observation arms by tumor cells PD-L1 staining status (negative, positive). (A–D) overall survival; (E–H) disease-free survival.

Our data showed that PD-L1 positivity was lower in *EGFR* mutant than in WT adenocarcinoma, although the differences did not reach statistical significance. In contrast, PD-L1 positivity at 1% and 25% TC cut-off was more frequent in patients with *KRAS* mutant NSCLC after multivariable adjustment. In CheckMate 057 [3], among the non-squamous NSCLC patients, subgroup analyses showed that nivolumab did not demonstrate survival benefit in patients with *EGFR*-mutated tumors compared with docetaxel. In contrast, survival benefit appeared to be greater in patients with *KRAS* mutant than WT tumors. However, it was unclear whether these differences were related to PD-L1 expression, as relationships between PD-L1 expression and *EGFR* and *KRAS* mutation status were not reported. In KEYNOTE-001 [2], PD-L1 expression was not different in *EGFR* mutant or WT tumors, while it was higher in patients with *KRAS* mutated (44.2%) compared with WT (26.8%) tumors. Among studies that used a validated PD-L1 antibody (Table 1), Cooper et al. [20] also observed lower frequency of PD-L1 positivity in *EGFR* mutated and higher positivity in *KRAS* mutant NSCLC, although the differences were not statistically significant due to low sample size. Calles et al. [21] recently reported that in *KRAS*-mutant lung cancer, strong PD-L1 expression was positively correlated with smoking status including pack-years. Interestingly, we found that *TP53* mutation, which is commonly regarded as a guardian of genomic stability and DNA repair, has no relationship with PD-L1 expression.

In summary, using the pooled cohort of four pivotal randomized adjuvant chemotherapy trials, we have demonstrated that PD-L1 expression is neither prognostic nor predictive of adjuvant chemotherapy benefit in early stage NSCLC patients, regardless of the cut-offs chosen.

Disclosure

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