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PD-L1 Expression in Patients with Non-small Cell Lung Cancer

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Abstract. *Aim: The aim of this study was to evaluate whether irradiation induces the expression of tumor programed cell death ligand 1 (PD-L1) in patients with non-small cell lung cancer (NSCLC). Patients and Methods: Seventeen patients with NSCLC who received chemoradiotherapy and underwent tumor resection and six patients whose pre-treatment biopsy specimens were available, were analyzed by immunohistochemistry for PD-L1 expression between September 2011 and June 2016 at the Institute of Biomedical Research and Innovation Hospital. Results: Among six patients for which pre-irradiation biopsy samples were available, the H-score for PD-L1 was reduced after irradiation following staining with two different antibody clones (SP28-8 and SP142). A PD-L1 H-score >5 with SP28-8 antibody (hazard ratio=6.46; 95% confidence interval=1.209-34.53; p=0.029) was a significant negative factor for duration of progression-free survival after curative operation or chemoradiation. Conclusion: We showed that tumor PD-L1 expression decreased in patients with NSCLC who received chemoradiotherapy and radiation resistance might be due to pre-treatment PD-L1 expression.*

Non-small cell lung cancer (NSCLC) is a leading cause of cancer-related death worldwide. Recent discoveries in biomedical research have provided a greater understanding of the druggable molecular basis of this disease (1). However, beyond targeting of these driver mutations, platinum-based, doublet chemotherapy remains the standard therapy.

In recent years, immune checkpoint inhibitors, such as those targeting programmed cell death protein 1 (PD1) or PD-ligand 1 (PD-L1), were introduced as another therapeutic option in this field with promising results (2). A monoclonal antibody against PD1, nivolumab, is now in clinical use and several PD-L1 antibodies are being evaluated in clinical trials (3). Furthermore, companion biomarkers that can predict the efficacy of these drugs are under exploration, such as immunohistochemistry (IHC) for intra-tumoral/microenviromental PD1 or PD-L1, infiltrating T-lymphocytes and somatic mutation burden.

Deng L *et al*. reported that PD-L1 expression increased on tumor cells ($CD45^-$), dendritic cells ($CD11c^+$) and macrophages ($CD11b^+F4/80^+$) after irradiation with 12 Gy in a TUBO tumor model, which may weaken irradiation-induced antitumor immunity (4). If this phenomenon is confirmed in the clinical setting, it would provide a rationale for including anti-PD-L1 therapy in the concurrent or adjuvant setting in combination with irradiation for the treatment of patients with NSCLC.

Here, we evaluated PD-L1 status of resected tumor tissue after induction chemoradiotherapy and also compared the PD-L1 status of pre- and post-treatment tumor tissues when specimens were available.

Materials and Methods

Patients (Cohort 1). Seventeen patients with NSCLC who received chemoradiotherapy and underwent tumor resection (one patient for brain metastasis) and six patients whose pre-treatment biopsy specimens were available were analyzed for PD-L1 IHC between September 2011 and June 2016 at the Institute of Biomedical Research and Innovation Hospital. This study was approved by the Institutional Review Board of our institution (Number 15-32). This study was conducted in accordance with the Declaration of Helsinki.

Cohort 2: Validation cohort. We retrospectively screened electronic medical records of 83 patients with advanced NSCLC who had undergone histological re-biopsies between January 2010 and October 2015 at in our Institute or Kobe City General Hospital.

PD-L1 IHC. Paraffin-embedded tumor tissues were sectioned at a thickness of 4 μm and the sections were then mounted on glass slides for PD-L1 immunohistochemistry (IHC). PD-L1 IHC was performed using SP142 and SP28-8 antibodies (Spring Biosciences, Pleasanton, CA, USA) for membranous staining of tumor cells. The H-score method was adopted to evaluate both the percentage and intensity of staining. The semi-quantitative H-score was determined by multiplying the percentage of stained cells by the intensity score (0, absent; 1, weak; 2, moderate; and 3, strong)

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Key Words: Immunohistochemistry, irradiation, non-small cell lung cancer, programmed cell death protein 1.

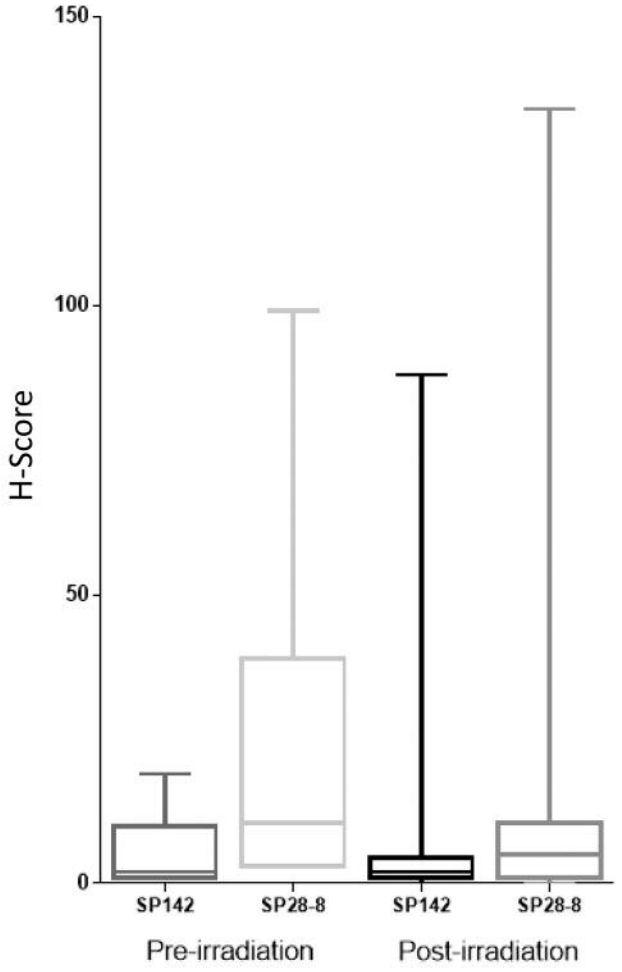

Figure 1. *H-Score for programmed cell death ligand 1 (PD-L1) of pre-irradiation biopsy and post-irradiation resected tumor specimens using two different antibody clones, SP142 and SP28-8. Boxes represent 75% quartile, median and 25% quartile. Lines represent upper and lower whiskers.*

leading to a maximum value of 300 corresponding to 100% of tumor cells positive for PD-L1 with an overall staining intensity score of 3. We did not definePD-L1 expression as positive or negative in this study.

Mutational analysis of the epidermal growth factor receptor (EGFR) and anaplastic lymphoma kinase (ALK) genes. A sufficient number of cancer cells for a pathological diagnosis (*i.e.* several hundred cells) were obtained from paraffin-embedded biopsy specimens by manual microdissection. Biopsy or surgical specimens were used for analysis of somatic *EGFR* mutations in exons 18-21, as previously described (5). IHC analysis of *ALK* expression was performed by fluorescent *in situ* hybridization with a mouse monoclonal antibody to ALK (ALK1; Dako, Japan), as previously described (6, 7). Seventeen patients were analyzed for ALK status, among which two had *ALK* rearrangements and 15 were wild-type for *ALK*.

Table I. *Characteristics of patients of cohort 1 (n=17).*

Characteristic		Value
Age, years	Range	44-80
	Median	66
Gender, n (%)	Male	11 (64.7)
	Female	6 (35.3)
Smoking status, n (%)	Non-smoker	3 (17.7)
	Smoker	14 (82.3)
	Former	9 (52.9)
	Current	5 (29.4)
Performance status, n (%)	0-1	17 (100)
Tumor histology, n (%)	Adenocarcinoma	11 (54.7)
	Squamous cell carcinoma	5 (29.4)
	LCNEC	1 (15.9)
Stage, n (%)	IIA	3 (17.7)
	IIB	3 (17.7)
	IIIA	6 (35.3)
	IIIB	5 (29.3)
EGFR, n (%)	Exon19 deletion	2 (11.8)
	Wild-type	12 (70.5)
ALK rearrangement, n (%)	Positive	1 (6.0)
	Negative	11 (64.7)
PD-L1 H-score	SP142	
	Range	0-88
	Mean	7.6
	Median	2
	SP28-8	
	Range	1-134
	Mean	13.0
	Median	5

LCNEC, Large-cell neuroendocrine carcinoma; *EGFR*, epidermal growth factor receptor; *ALK*, anaplastic lymphoma kinase; PD-L1, programmed cell death ligand 1; SP142/SP28-8, antibody clone used.

Statistical analysis. Progression-free survival (PFS) was calculated from the date of diagnosis until the date of disease recurrence or death for all enrolled patients. To evaluate risk factors associated with PFS, a Cox proportional hazards regression model was used. PFS curves were generated using the Kaplan–Meier method. The log-rank test was used to evaluate differences between PFS curves. Statistical analyses were performed using GraphPad Prism (ver. 6; GraphPad Software, Inc., San Diego, CA, USA) and 'R' (R Foundation for Statistical Computing, Vienna, Austria) software programs.

Results

The characteristics of the 17 enrolled patients are shown in Table I. All the patients were Japanese and underwent tumor resection after induction chemoradiotherapy. The patients had a median age of 66 (range=44-80) years and comprised of 11 (64.7%) men and six (35.3%) women. Patient characteristics with tumor stage and somatic mutation status are shown in Table I.

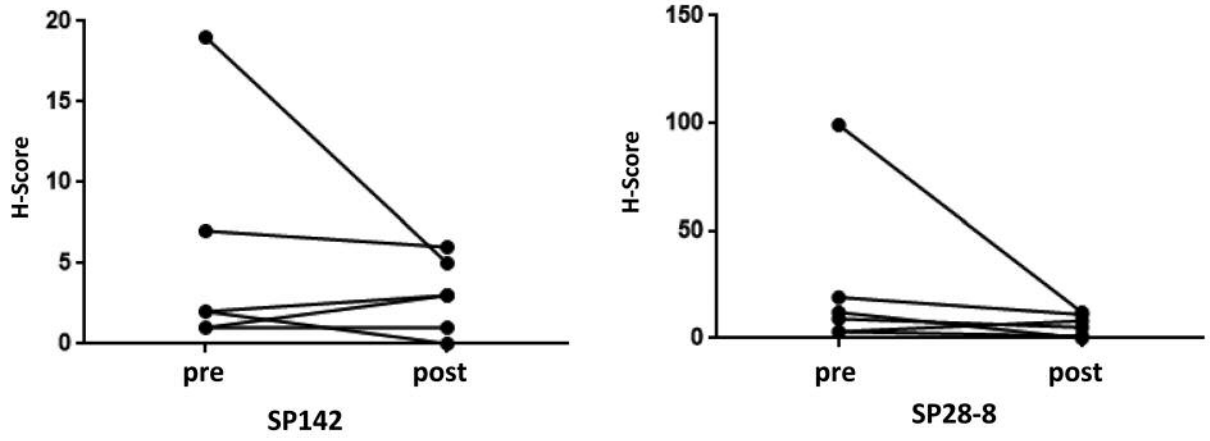

Figure 2. *Changes in H-score for programmed cell death ligand 1 (PD-L1) between pre- and post-irradiation samples among six patients using two different antibody clones SP142 (A) and SP28-8 (B).*

Table II. *Characteristics of patients of Cohort 2 (validation) (n=83).*

Characteristic		Value
Age, years	Range	26-84
	Median	74
Gender, n (%)	Male	40 (48.2%)
	Female	43 (51.8%)
Smoking status, n (%)	Non-smoker	27 (67.5%)
	Smoker	56 (22.5%)
Tumor histology, n (%)	Adenocarcinoma	73 (89.0%)
	Squamous cell carcinoma	8 (9.6%)
	LCNEC	2 (1.4%)
Genotype, n (%)	*EGFR*-mutated	63 (75.9%)
	ALK rearrangement	3 (3.6%)
	Wild-type	17 (20.5%)
PD-L1 H-score	SP142	
	Range	0-91
	Mean	3
	Median	3
	SP28-8	
	Range	1-150
	Mean	9
	Median	9

LCNEC, Large-cell neuroendocrine carcinoma; *EGFR*, epidermal growth factor receptor; *ALK*, anaplastic lymphoma kinase; PD-L1, programmed cell death ligand 1; SP142/SP28-8, antibody clone used.

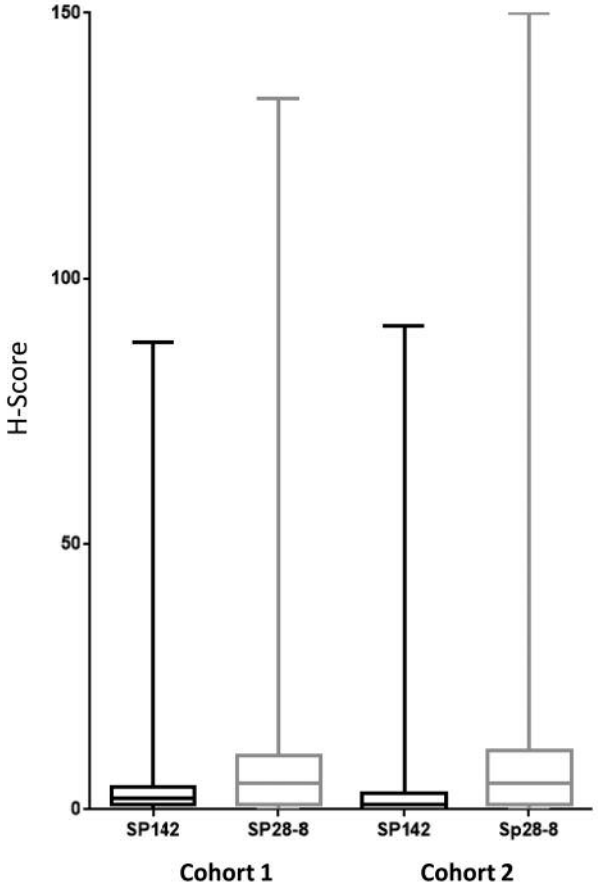

Figure 3. *H-Score for programmed cell death ligand 1 (PD-L1) of cohort 1 (post-irradiation biopsy) and cohort 2 using two different antibody clones, SP142 and SP28-8.*

The H-scores for PD-L1 are listed in Table I and Figure 1. The H-score tended to be higher following staining with the SP28-8 clone antibody than with the SP142 clone antibody. The median score (range) of PD-L1 expression was 2 (0-88) and 5 (1-134) for the SP142 and SP28-8 clones, respectively. Among the six patients for which a pre-irradiation biopsy sample was available, the H-score for PD-L1 was reduced after irradiation, regardless of the antibody clone used (Figure 2).

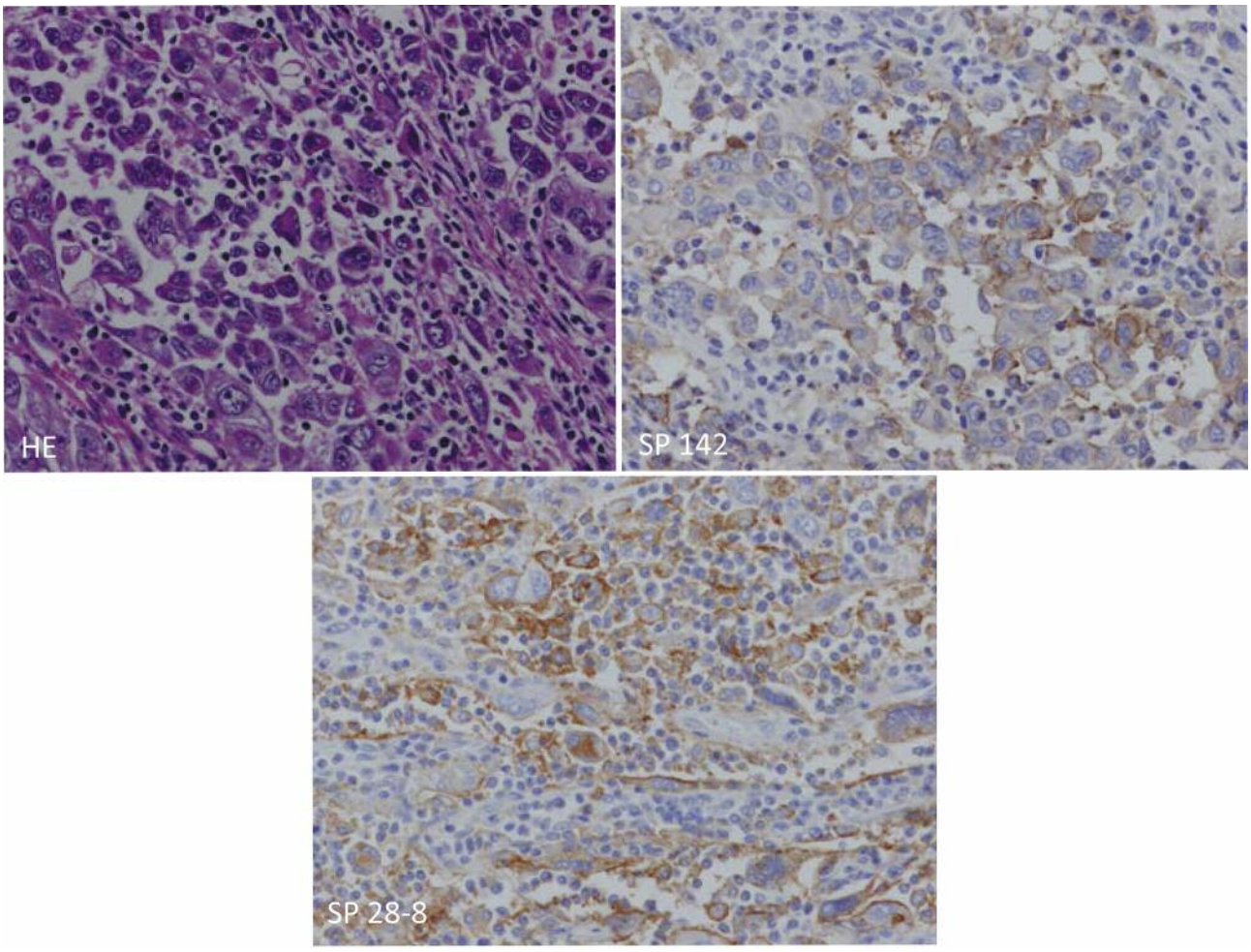

Figure 4. *Haematoxylin and eosin (H&E) staining and immunohistochemistry for programmed cell death ligand 1 using clones SP142 and SP28-8 for the specimen with the highest H-score in this cohort.*

The characteristics of the 83 enrolled patients of the validation cohort are shown in Table II. The median score and range of PD-L1 expression was 3 (0-91) and 9 (0-150) for the SP142 and SP28-8 clones, respectively. There was no difference in H-score between the two cohorts by *t*-test, regardless of the antibody clone used (Figure 3). Representative staining of tissue with the highest H-score in this study (88 for SP142 and 134 for SP28-8) is shown in Figure 4.

Among the 17 patients of Cohort 1, the median duration of PFS of patients that were PD-L1-negative was significantly longer than that of those that were PD-L1-positive (Figure 5A and B). A PD-L1 H-score >5 using the SP28-8 clone (hazard ratio(HR)=6.46, 95% confidence interval(CI)=1.209-34.53; p=0.029) was a significant negative factor that affected the duration of PFS following curative operation or chemoradiation. Multivariate analysis could not be carried out because of the small sample size.

Discussion

In this study, we evaluated whether irradiation in patients with NSCLC induces the expression of PD-L1 on tumor cells but instead found a reduction in PD-L1 expression after induction chemoradiotherapy.

This result was unlike a model proposed in a previous report (4), in which expression of PD-L1 was up-regulated in the tumor and tumor microenvironment after ionizing irradiation. However, this finding is in line with the "abscopal effect" (8), which is the concept that localized radiation therapy is able to elicit out-of-target tumor responses. This process is likely mediated by the immune system, with dendritic cells, T-regulatory cells and suppressor cells acting as critical mediators (9-11). Our result may demonstrate that PD1/PD-L1 immunomodulation contributes to this abscopal effect (12).

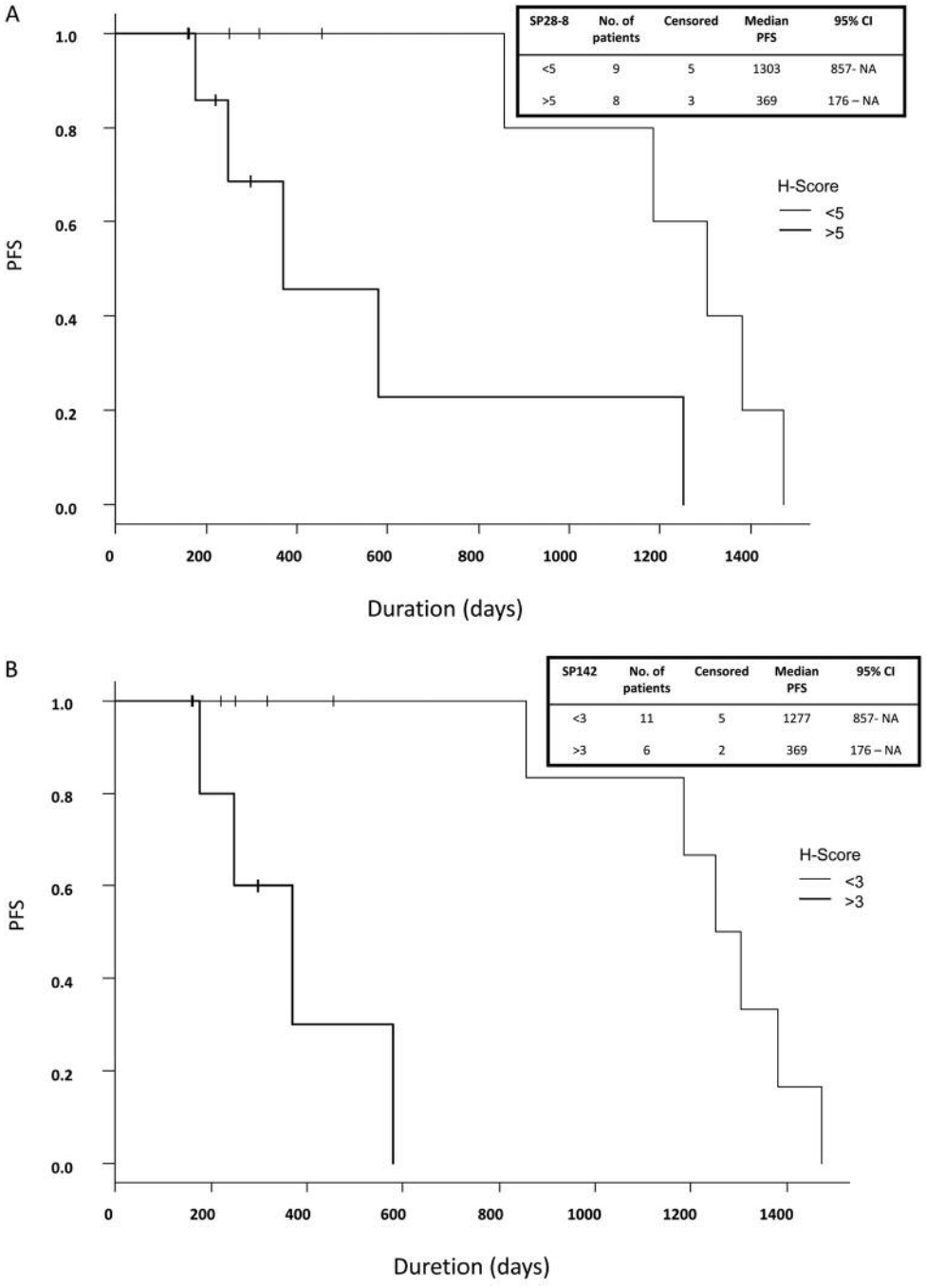

Figure 5. *Progression-free survival (PFS) by Kaplan–Meier analysis of 17 patients was related to their post-therapy H-Score for programmed cell death ligand 1 (PD-L1) using clones SP28-8 (A) and SP142 (B). The difference between the groups was evaluated with the log-rank test (A: p=0.0138; B: p=0.0046). NA: Not applicable.*

The notion that local up-regulation of the PD1–PD-L1 axis suppresses radiation-induced immune responses and limits the full expression of antitumor immunity thereby facilitating relapse may be correct. However, it may depend on baseline expression of the PD1–PD-L1 axis, not on expression changes induced by ionizing irradiation. This is corroborated by our finding that PFS was significantly longer in PD-L1-negative patients. In other words, ionizing irradiation itself might be an immunomodulator that induces peripheral T-cell repertoires.

Many studies to confirm biomarkers of the response to

drugs targeting the PD1–PD-L1 axis are currently being performed. For instance, anti-PD1 therapy has been reported to be correlated with the nonsynonymous mutation burden in NSCLC (13). However, it is currently unclear whether the first candidate biomarker, immunological analysis of PD-L1, should be an absolute selection criterion for therapy. Sunshine *et al*. reported that the response rate to PD-L1 blockade in patients with PD-L1-positive tumors was 48% compared to 15% in those with PD-L1-negative tumors (14). According to these results, additional information, such as genetic backgrounds and change in gene expression after ionizing irradiation, will benefit this field of study.

The limitations of our study include a small sample size and the retrospective nature of the study. Nonetheless, we were able to show a reduction in PD-L1 expression after induction chemoradiotherapy, albeit from samples of only six patients, and a genuine up-regulation of the PD1–PD-L1 axis that may be a factor in therapeutic resistance. A prospective study with stricter criteria for the selection of patients is needed to overcome these limitations.

In conclusion, we showed that tumor PD-L1 expression in patients with NSCLC who received chemoradiotherapy decreased and radiation resistance might be due to pre-treatment expression of PD-L1. This phenomenon provides a rationale for the use of immune checkpoint inhibitors as combination therapy with irradiation or adjuvant therapy for NSCLC.

Compliance with Ethical Standards

All procedures performed in studies involving human participants were in accordance with the ethical standards of the Institutional Committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Funding

None.

Conflicts of Interest

The Authors declare they have no conflicts of interest in regard to this study.

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