Distribution of PD-L1 expression in diverse cancer types: experience with over 10,000 cases

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Abstract

Background: Blocking the programmed death-1 (PD-1) pathway has clinical benefit in metastatic cancer and has led to the approval of the mAbs to treat several cancer types. Expression of PD-1 ligand (PD-L1) on the cell surface of tumor cells is generally associated with a higher likelihood of response to PD-1 blockade in multiple studies. We analyzed distribution of PD-L1 in a wide variety of tumor types, studied underlying mechanisms of expression and compared methods of detection.

Methods: 10,187 tumor samples were tested for the expression of PD-L1 (SP142 antibody, Spring Biosciences). Expression (IHC) of PD-L1 in a selected number of cases was compared to 3 additional monoclonal antibodies (SP263, Ventana; 22c3 and 28-8 DAKO). PD-L1 gene amplification was calculated using the next generation sequencing (NGS) data.

Results: Using the ≥2+ intensity and ≥5% IHC threshold, PD-L1 was detected with the highest frequency in thymic carcinoma (63%), followed by lymphomas (42%), head and neck squamous carcinoma (35%), anal carcinoma (31%), non-small cell lung cancer (30%), thyroid carcinoma (29%), bladder carcinoma (22%), cervical carcinoma (20%), melanoma (19%), and esophageal and esophagogastric junction carcinoma (15%). Significant differences between genomically defined cancer subtypes were observed (e.g. 4-fold more frequent PD-L1 expression in MSI-H colorectal and endometrial carcinomas vs. MSS counterparts). Analytical comparison of 4 different, yet well-defined Abs used in numerous clinical trials showed overall concordance of >90%, irrespective of the thresholds employed. High copy number (≥8) of PD-L1 gene was identified in 1/2 DLBCL, 1/3 thymic carcinoma and additionally in a small percentage of breast carcinoma (4%), non-small cell lung cancer (2%) and ovarian carcinoma (1%).

Conclusions: Targetable PD-L1 protein expression was identified in numerous cancer types, including some rare but highly overexpressing types not previously recognized. Threshold determination for predictive purposes remains challenging, but concordance between 4 antibodies was superb. High PD-L1 gene amplification (alone or co-amplification with JAK2 gene) is detected in a minority of carcinomas, and additional methods of detection (e.g. in-situ hybridization) may be needed.

Introduction

Programmed cell death protein 1 (PD-1) and its ligand the programmed death ligand (PD-L1) are among the most important checkpoint proteins that mediate tumor-induced immune suppression through T-cell downregulation (1). Their overexpression has been described in various solid tumors with marked clinical therapeutic effects due to the checkpoint blockade (2). PD-L1 status is usually determined by immunohistochemistry (3,4). For that purpose FDA has recently approved DAKO antibody (PD-L1 IHC 22C3 pharmDx) as an aid in identifying NSCLC patients that are candidates for treatment with pembrolizumab.

Materials and Methods

>14,000 tumor samples were tested for the expression of PD-L1 (clone SP142, Spring Biosciences). Expression (IHC) of PD-L1 in a selected number of cases was compared to 3 additional monoclonal antibodies (SP263, Ventana; 22c3 and 28-8 DAKO). Cases were considered positive ≥5% of the tumor cells exhibited membranous positivity with 2+/3+ intensity (5, 6). In selected cases, *PD-L1* gene amplification was explored using the next generation sequencing (NGS) data and in-situ hybridization assays (FISH and CISH). A 586kb probe was designed to cover the JAK2/PD-L1/PD-L2 gene region at 9p24.1 (chr9:4985240-5571285). A second probe was designed to cover the peri-centromeric region of chromosome 9 (chr9:38079360-38446085) as a chromosome copy number control. Both probes were designed to be free of repetitive sequences and synthesized using Agilent's oligo-based SureFISH technology. The 9p24.1 and peri-centromeric probes were labeled with Texas Red and fluorescein fluorochromes, respectively.

Results (updated)*

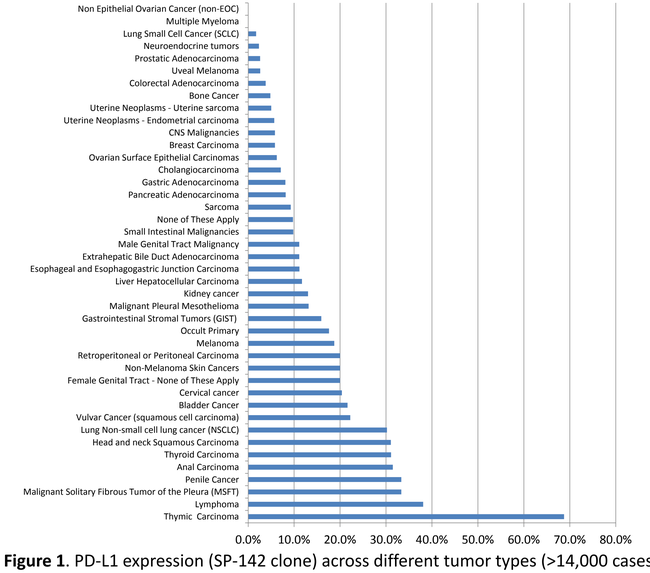

Figure 1. PD-L1 expression (SP-142 clone) across different tumor types (>14,000 cases)
* Since abstract submission, >4,000 additional cases were tested and were added to the presentation.

Results Continued (updated)

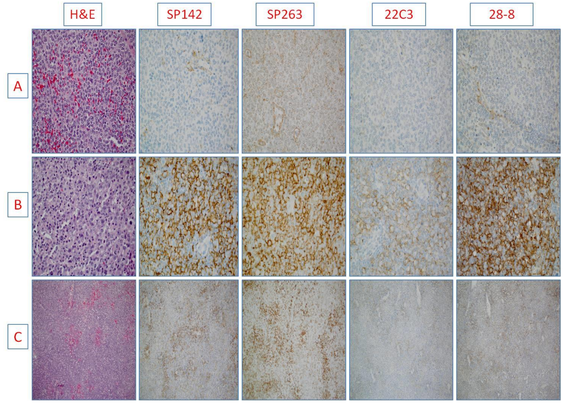

Figure 2. Concordance in IHC expression (2+, ≥5% positive tumor cells) of 4 different anti-PD-L1 clones in malignant lymphomas: (A): Case of marginal zone lymphoma: **Negative by all 4 clones**; (B): Peripheral T-cell lymphoma: **Positive by all 4 clones**; (C): Diffuse Large B-cell lymphoma (**variable positivity**); copy number variation analysis by NGS did not detect underlying *PD-L1/PD-L2/JAK2* alterations at 9p24.

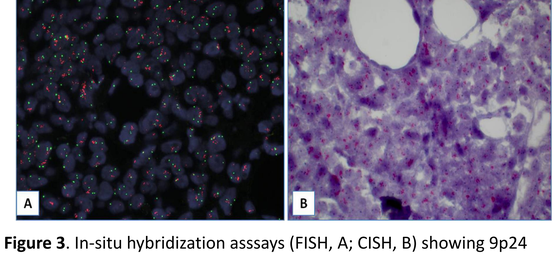

Figure 3. In-situ hybridization asssays (FISH, A; CISH, B) showing 9p24 amplification (*PD-L1/PD-L2/JAK2*) in a case of diffuse large B-cell lymphoma with PD-L1 overexpression by IHC (not shown). *PD-L1* gene amplification was also observed in a small subset of tested tumors (4% breast, 2% NSCLC, 1% ovarian cancer).

Results Continued (updated)

		SP142		
		-	+	Total
SP263	-	21	1	42/44
	+	1	21	
	% Conc.	**95%**	**95%**	**95%**
22c3	-	25	0	48/50
	+	2	23	
	% Conc.	**93%**	**100%**	**96%**
28-8	-	22	1	41/45
	+	3	19	
	% Conc.	**88%**	**95%**	**91%**

Table 1. Concordance between SP142 and other three anti-PD-L1 antibodies' clones tested on a subset of cases including NSCLC, melanoma, breast, colorectal and renal cell carcinomas.

Conclusions

- PD-L1 protein expression was identified in diverse cancers, including some rare types previously unknown.
- Threshold determination for predictive purposes remains challenging, but concordance between 4 tested antibodies was high (overall 91%).
- *PD-L1 gene* amplification (alone or co-amplification with *PD-L2* and *JAK2* genes) is detected in a minority of cancers, and different methods of detection (NGS vs. in-situ hybridization) may be needed. Additional studies are needed to determine if gene amplification is predictive of response.

References

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