



Relevance of PD-L1 expression status in thymic malignancies

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The anticancer immune response can be described as a cyclic cancer-immunity cycle in which tumour neoantigens are produced, T cells are primed and activated, and the tumour environment is infiltrated by effector T cells and other immune cells, ultimately leading to apoptosis and death in target cancer cells. In an optimal setting this leads to eradication of the tumoral cells. In cancer patients the equilibrium of the cancer-immunity cycle is disrupted, for instance due to specific stimulatory or inhibitory signals modulating T cell activation which may positively or negatively regulate each step of this cyclic process. The tumours can create negative feedback on immune reaction in this loop, for instance by increasing the inhibition by immune checkpoints on lymphocytes.

The defined goal in the emerging field of cancer immunotherapy is to disconnect negative feedback loops and to re-establish an independent and self-supporting cycle of cancer immunity (1).

The signalling pathway involving the immune checkpoint molecules programmed death-1 (PD-1) (CD279) and its ligands—programmed cell death ligand 1 (PD-L1) (CD274) as well as programmed cell death ligand 2 (PD-L2) (CD272) figures prominently in T cell regulation (2). PD-1 is upregulated on activated T cells and can inhibit cytotoxic T cell effector functions by binding with PD-L1 (3). PD-L1 is expressed and upregulated in various tumour types, such as in melanoma, colon, lung and breast tumours and inhibits a T cell response to the tumour, making it an appealing target for immune checkpoint blockade therapy (2,3). In the field of non-small cell lung cancer PD1- or PD-L1-mediated

signalling can be inhibited by atezolizumab, pembrolizumab or nivolumab and further agents in clinical development (4).

As the thymus is central in T cell maturation, a neoplasm of thymic epithelial cells might lead to abnormally conditioned T cells which often account for autoimmune disorders, for instance myasthenia gravis, connective tissue diseases or blood disorders (5). Thymic epithelial tumours are rare thoracic tumours, with an annual incidence of 1.3–3.2 per million, and include thymic carcinomas and the more frequent thymomas. Thymomas are subclassified into different subtypes depending on their morphology and aggressiveness (type A, AB, B1–B3). For the staging of thymomas mostly a modified Masaoka-Koga system is used. The Masaoka-Koga staging system is also used for thymic carcinomas (type C). Here, the squamous cell carcinoma subtype prevails and surgical resection is the only treatment option for long-term survival. Surgery is also the first treatment option in early-stage thymoma. In advanced or metastasized stages multimodal therapy approaches, including surgery, chemotherapy and radiotherapy, are used. As there is a lack of independent and reliable prognostic and predictive molecular markers, the field of personalized medicine for thymic malignancies is in the early stages of development (3,6,7).

Therefore, the authors of the article “Immunohistochemical status of PD-L1 in thymoma and thymic carcinoma” are commended for investigating whether PD-L1 could be a possible therapeutic target in patients with thymic malignancies, which may open novel avenues for cancer immunotherapy (3). The analysis was conducted at

the National Cancer Center Hospital in Tokyo, Japan, and included 139 retrospectively collected evaluable patient samples with immunohistochemically confirmed primary thymoma (101 samples) or thymic carcinoma (38 samples). The tumours were resected between 1973 and 2009; resection margin status ranges from R0 to R2. Median patient age was 58 years (range from 25 to 84), 38% of patients were male. The majority did not receive preoperative therapy (96%) or adjuvant treatment after resection (81%). All WHO classification stages were included, and the majority of tumours were type AB (type A: 6%, type AB: 34%, type B1: 12%, type B2: 17%, type B3: 4%, type C 27%).

Tissue microarrays (TMAs) were generated from the formalin-fixed paraffin embedded (FFPE) samples. In order to meet the challenge of heterogeneity within the tissue two random samples from each specimen were taken. Staining was performed with a rabbit monoclonal PD-L1 antibody (clone E1L3N, Cell Signaling Technology, Danvers, MA, USA), which had been validated previously. Moreover, a PD-1 antibody (NAT105, Abcam, Cambridge, UK) was used. Evaluation of the stained samples was conducted by two independent observers and included staining percentages for PD-L1 (0–100%) and intensity ranging from 0 to 3 (0= negative, 1= very weak, 2= moderate, 3= strong) in tumour cells. In addition, PD-1 expression was assessed for tumour infiltrating lymphocytes (TILs). Multiplication of the percentage of tumour area stained with staining intensity resulted in a PD-L1 and PD-1 expression score ranging from 0 to 300.

Taking the specificity of the antibodies into account, a detection cut-off score of 3 (1%) was applied for the immunohistochemical detection of PD-L1. Results show that 23% of thymomas and 70% of thymic carcinomas were positive for PD-L1 ($P < 0.001$), which could be detected on tumour cells. Between thymomas and thymic carcinomas, the likelihood ratio of a positive result was 3.25, and likelihood ratio of negative result was 0.38. The average PD-L1 expression scores of thymomas and thymic carcinomas were 5.98 (standard deviation 17.85) and 41.21 (standard deviation 50.28) ($P < 0.001$), whereas the mean PD-L1 expression scores of thymomas and thymic carcinomas were 0 and 25. Expression of PD-1 could be shown on TILs, not on tumour epithelial cells with a cut-off score of 3 (1%). In thymic carcinoma 23 patients (62%) were positive for PD-1 and 14 patients (38%) were negative. No correlation could be shown between PD-1 and PD-L1 expression.

When PD-L1 expression status was associated with patient characteristics and pathological features Katsuya *et al.* could show, that a WHO classification Type C (thymic carcinoma) significantly correlated with PD-L1 positivity ($P = 0.006$). PD-L1 expression could not be associated with primary tumour size, Masaoka-Koga stage, neoadjuvant therapy, age or sex. Regarding survival no significant correlation between overall survival (OS) and PD-L1 positivity resp. negativity could be shown. It would be interesting whether a higher percentage of positive cells would deliver better correlations.

As 70% of thymic carcinomas and 23% of thymomas were positive for PD-L1 it is evident that PD-L1 positivity correlates significantly with WHO classification (thymoma or thymic carcinoma). If PD-L1 is found to be a predictive factor for anti PD-1/PD-L1 treatment in this setting, the obtained data might suggest selecting thymic carcinoma patients; however, treatment effect was not investigated in the current study (3).

The data presented is consistent with a previous study conducted by Padda *et al.* showing that high PD-L1 expression was found in 68.1% of thymic epithelial tumours compared to 17.6% in normal thymus control samples (8). However, in several other studies, there are discrepancies concerning PD-L1 expression, as for instance Marchevsky *et al.* showed PD-L1 expression in 90% of nonneoplastic thymi, 92% of thymomas and 50% of carcinomas (9). This might be caused by differences in staining procedures, antibody clones, assays and scoring systems as well as PD-L1 heterogeneity. Perhaps there are also differences due to various ethnic backgrounds and age subgroups.

Katsuya *et al.* also address the question of distinct PD-L1 antibody clones and compare the PD-L1 clone 5H1 with the clone E1L3N, which they used in this study. In comparison to 5H1 the clone E1L3N targets intracellular domains of PD-L1. This might result in different staining/scoring outcomes. However, it is still unclear which cellular PD-L1 domain needs to be targeted to best predict treatment response. As the clone 5H1 was not available, Katsuya *et al.* validated E1L3N affinity with A549 cells with or without knockdown of PD-L1 beforehand. Moreover, E1L3N exclusively stains tumour cells rather than other PD-L1 positive cells, such as lymphocytes, dendritic cells or tissue stroma cells, and is very suitable for quantitatively evaluating expression intensity (3).

In summary, the study by Katsuya *et al.* clearly shows that thymic malignancies express PD-L1, probably varying in intensity between the subtypes, and suggests that there

may be a role for targeted immune therapies in this setting. As 38 thymic carcinoma patients were included in this study an enlargement of sample size is desirable in future studies. Moreover, as thymic carcinomas are known to be very heterogeneous, a comparison of generated TMAs with the corresponding whole slides might deliver insightful findings in terms of PD-L1 distribution. In addition, the immunohistochemically evaluated expression status needs to be correlated with treatment response to anti PD-L1 drugs. Cancer immunotherapy targeting PD-1/PD-L1 may very well enrich treatment options in the field of thymic malignancies. To date there have been few reports of cases and phase-I/II data on PD1-/PDL1-inhibition in this setting. For example, Yang *et al.* reported that anti-PD-1 therapy (pembrolizumab, 2 mg/kg, every 3 weeks) administered to a 68-year-old female with metastatic squamous cell carcinoma of the thymus resulted in complete remission after eight cycles. To our knowledge PD-1/PD-L1 status was not evaluated. In this case no severe adverse events occurred during anti-PD-1 treatment (10). But in some trials, there is a high rate of autoimmune side effects (11). An analysis of PD-1/PD-L1 expression status might help to stratify patient groups and predict therapy response.

In summary PD-1/PD-L1 status seems to be important for stratification of thymic tumours and may be relevant for choice of systemic therapy. To address these questions, further well-designed retrospective, but also prospective, trials like the trial from Katsuya and colleagues are needed, with a focus on the link between expression status and therapy response.

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References

1. Chen DS, Mellman I. Oncology Meets Immunology: The Cancer-Immunity Cycle. *Immunity* 2013;39:1-10.
2. Yuan J, Hegde PS, Clynes R, et al. Novel technologies and emerging biomarkers for personalized cancer immunotherapy. *J Immunother Cancer* 2016;4:3.
3. Katsuya Y, Fujita Y, Horinouchi H, et al. Immunohistochemical status of PD-L1 in thymoma and thymic carcinoma. *Lung Cancer* 2015;88:154-9.
4. Sheng Z, Zhu X, Sun Y, et al. The efficacy of anti-PD-1/PD-L1 therapy and its comparison with EGFR-TKIs for advanced non-small-cell lung cancer. *Oncotarget* 2017. [Epub ahead of print].
5. Engels EA. Epidemiology of thymoma and associated malignancies. *J Thorac Oncol* 2010;5:S260-5.
6. Girard N, Ruffini E, Marx A, et al. Thymic epithelial tumors: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2015;26:v40-55.
7. Marx A, Chan JK, Coindre JM, et al. The 2015 WHO Classification of Tumors of the Thymus: Continuity and Changes. *J Thorac Oncol* 2015;10:1383-95.
8. Padda SK, Riess JW, Schwartz EJ, et al. Diffuse High Intensity PD-L1 Staining in Thymic Epithelial Tumors. *J Thorac Oncol* 2015;10:500-8.
9. Marchevsky AM, Walts AE. PD-L1, PD-1, CD4, and

- CD8 expression in neoplastic and nonneoplastic thymus. Hum Pathol 2017;60:16-23.
10. Yang Y, Ding L, Wang P. Dramatic response to anti-PD-1 therapy in a patient of squamous cell carcinoma of thymus with multiple lung metastases. J Thorac Dis 2016;8:E535-7.
 11. Cho J, Yoo KH, Lee H, et al. A phase II study of pembrolizumab for patients with previously treated advanced thymic epithelial tumor. J Clin Oncol 2017;35:abstr 8521.

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