

## IMMUNOHISTOCHEMICAL EXPRESSION OF PD-L1 IN SOLID TUMORS

PETRA RADULOVIĆ<sup>1</sup> and BOŽO KRUŠLIN<sup>1,2</sup>

<sup>1</sup>Department of Pathology, Sestre milosrdnice University Hospital Center, Zagreb, Croatia

<sup>2</sup>School of Medicine, University of Zagreb, Zagreb, Croatia

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### Summary

Recent clinical trials have demonstrated that it is possible to induce durable remission in several tumors (non-small cell lung cancer (NSCLC), melanoma, squamous cancer of head and neck, renal cell carcinoma, Hodgkin lymphoma, colorectal cancer) by blocking the PD-1/PD-L1 (programmed death-1/programmed death-ligand 1) axis with anti-PD-1 or anti-PD-L1 antibodies and that an objective clinical response was closely associated with immunohistochemical PD-L1 expression in tumor cells. Because immunohistochemistry is widely accepted and used method for PD-L1 assessment it is important to define criteria for selecting patients who are candidates for immunotherapy and can benefit from it.

KEY WORDS: *PD-1/PD-L1 pathway, immunotherapy, immunohistochemistry, solid tumors*

### IMUNOHISTOKEMIJSKA IZRAŽENOST PD-L1 U SOLIDNIM TUMORIMA

#### Sažetak

Posljednjih godina klinička istraživanja su pokazala da je moguće postići trajniju remisiju kod nekoliko vrsta solidnih tumora (karcinom pluća ne-malih stanica (NSCLC), melanom, pločasti karcinom glave i vrata, renalni karcinom, urotelni karcinom, Hodgkin limfom, kolorektalni karcinom) blokirajući aktivaciju PD-1/PD-L1 (engl. *programmed death-1/programmed death-ligand 1*) signalnog puta primjenjujući anti-PD-1 i anti-PD-L1 protutijela. Učinak terapije usko je povezan s izraženošću PD-L1 u tumorskim stanicama. Iako su mehanizmi kojima tumorske stanice izražavaju PD-L1 dobro poznati, studije su do sada pokazale da samo manji broj pacijenata može imati koristi od PD-1/PD-L1 imunoterapije, vezano uz izraženošću PD-L1 na tumorskim stanicama. Stoga je važno odrediti kriterije za odabir pacijenata koji mogu imati koristi od imunoterapije.

KLJUČNE RIJEČI: *PD-1/PD-L1 signalni put, imunoterapija, imunohistokemija, solidni tumori*

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### INTRODUCTION

As cancer therapy has recently focused on immunotherapy, driving efforts on immune checkpoint inhibitors in order to enhance the immune response against tumor cells, pathologists are challenged to bring results that will help in choosing patients who will benefit from therapy. A large number of studies is focused on Programmed cell death 1 (PD-1) and its ligand Programmed cell death ligand 1 (PD-L1/B7-H1/CD274) because of the involvement of this pathway in downregulating intensity and duration of

T-cells immune responses. Once activated, PD-1/PD-L1 pathway inhibits T-cells proliferation and survival as well as the effect or functions such as cytotoxicity and cytokine release (1).

PD-1 is a member of the immunoglobulin gene family and several studies demonstrated its expression on the surface of activated T cells, activated B cells, regulatory T-cells (Treg) and natural killer cells (NK). It has two ligands, PD-L1 and PD-L2 and when the T-cell receptor PD-1 binds to its ligands on antigen presenting cells (APC), the inhibitory pathway is activated leading to T-cells suppression (2).

PD-L1 (B7-H1 or CD274) is a cell surface glycoprotein that is mainly expressed in placenta, tonsil and retina, all implicated in immune tolerance mechanism: protein can also be expressed on hematopoietic cells (dendritic, myeloid, T and B cells), non-hematopoietic cells and on tumor cells (3). PD-L1 mRNA is expressed in almost all human tissues but cell membrane protein expression is confined to specific groups of cells. Then, it is conceivable that PD-L1 mRNA regulation is normally depending on post-transcriptional regulation. On the other side, the protein can be expressed on different types of cancer cells (4). Antibodies that target either PD-1 or PD-L1 will block this ligand-receptor interface, thereby allowing T cells to attack the tumor and increase antitumor immune response. Recent clinical trials have demonstrated that it is possible to induce durable remission in several tumors (non-small cell lung cancer (NSCLC), melanoma, renal cell carcinoma) by blocking the PD1/PD-L1 axis with anti-PD-1 or anti-PD-L1 antibodies and that an objective clinical response was closely associated with immunohistochemical PD-L1 expression in tumor cells (5). Immunohistochemistry is widely accepted and used method for PD-L1 assessment. However, criteria for selecting patients who are candidates for immunotherapy, and can benefit from it, are still debated and are very diverse depending on histological type of tumor as well as on antibody used (further depending on drug planned to use). There are some specific issues related to PD-L1 expression and results of evaluation. Recent studies focused on some important patients aspects like age, weight and microbiota emphasizing how they can deeply influence immune reaction to cancer and therefore, the response to immunotherapy (6). On the other hand, there are many factors that can influence PD-L1 expression evaluation. It is important, for now, that every anti PD-1/PD-L1 agent requires its own companion immunohistochemical diagnostics, meaning that for each drug there is specific antibody clone for immunohistochemistry as well as evaluation protocol.

One among many questions concerning PD-L1 immunohistochemistry evaluation is which kind of sample should be collected: small biopsy or surgical resection material. Formalin fixed paraffin embedded tissue is appropriate for analysis as well as fresh tissue specimens. Rehman JA et al. study shows that single paraffin block with surgi-

cally resected tumor tissue may be representative of the larger tumor and that 90% of the heterogeneity is presented in a single slide, at the millimeter level (7). On small biopsy fragments, like bronchial or transthoracic biopsies, the expression of PD-L1 can be overestimated but more often underestimated compared to the expression of large tumor sections and certain patients may not receive sufficient treatment. Also the proportion of tumor and stromal cells can differ from one tumor to another and the percentage of tumor cells can vary from 1 to 2% up to almost 100% (8).

Because PD-L1 is tumor marker represented as biological continuum of protein expression from very low levels through moderate to very high levels, result of immunohistochemical evaluation is percentage of positive cells, where positivity is seen as membrane staining, meaning that cytoplasmic staining is excluded from positive result. Most recent studies do not integrate intensity reporting into pathology report (9). Analysed cells can be tumor cells or tumor associated immune cells or both. In tumors like melanoma, colorectal cancer or NSCLC analysed cells are tumor cells while analysing urothelial carcinoma staining of tumor associated immune cells has to be found for claiming positive result. Clinical trials show that different drugs and different tumors have different cutoffs defining positive and negative groups of patients. That scale of positivity makes PD-L1 imperfect marker. So the question is how to define a threshold for positive PD-L1 labeling on biopsy tissue samples, taking into account that certain patients respond to treatment targeting PD-L1/PD-1, despite low or absent immunoreactivity of this biomarker (10). For different studies and antibody clones used, the threshold to call PD-L1 expression positive by immunohistochemistry differs. While in some studies, a threshold of 50% for tumor cells (like NSCLC) or of 5% for tumor associated immune cells (like for urothelial carcinoma) was related to optimal response to therapy, other studies set a lower threshold of 1% of positive tumor cells (like melanoma) (11,12). In comparison, tumor histologies currently thought to be less responsive to immune checkpoint inhibition, such as colorectal cancer and sarcoma, showed tumor PD-L1 expression ranging between 12%–53%, indicating that tumor PD-L1 immunohistochemical expression is not the sole determinant of which tumors histologies respond to PD-1/PD-L1 directed inhibition (13).

PD-L1 is a heterogeneous and dynamic marker, meaning that within the same tumor expression can vary according to tumor differentiation (5). In tumor cells PD-L1 expression can change during and after treatment influenced by the administered drugs and by immune state of the organism. Indeed, chemotherapy or target therapy may induce PD-L1 expression in immune therapy-naive tumors (14).

Study conducted on cell lines from non-small cell lung carcinoma (NSCLC) showed the effects of different chemotherapies on PD-L1 expression. It has been shown that doxorubicin can down-regulate membranous PD-L1 expression on cancer cells and that etoposide and paclitaxel are able to induce PD-L1 expression on cancer cells (15).

Another question is whether primary tumors differ in PD-L1 expression from their corresponding metastases. Jilaveanu et al. conducted a study on a series of primary clear cell renal cell carcinoma and matched metastases which showed greater PD-L1 expression in metastatic tumor than primaries (16). Another recent study has also demonstrated difference in PD-L1 expression between primary and metastatic of a series of primary clear cell RCC and corresponding metastases (2). Studies like these show that it might be significant to assess PD-L1 expression on the primary tumor as well as on metastatic lesion.

## CONCLUSION

Concerning all above mentioned it is clear that many further investigations are needed in order to establish standard and reproducible criteria for PD-L1 detection and to better select the target patients acceptable for anti PD-1 or anti PD-L1 therapy.

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*Corresponding author: Petra Radulović, Clinical Department of Pathology Ljudevit Jurak, Sestre milosrdnice University Hospital Center, Vinogradska 29, Zagreb, Croatia. e-mail: pradulovi@gmail.com*