Detection of PD-L1 RNA expression in immunohistochemically negative patients. Are the negatives a heterogeneous group?

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Urothelial carcinoma and PD-L1

Immune-checkpoint inhibition is an already well established therapy for advanced urothelial carcinoma.

Identification of patients who will respond to PD-1 or PD-L1 blockade is of paramount importance for clinical decision making.

Immunohistochemically determined PD-L1 status (currently) the only one validated biomarker for response prediction.

**BUT:** There are patients who respond to immune checkpoint therapy despite lack of PD-L1 expression.

We investigated PD-L1 RNA expression in situ in metastasized urothelial bladder cancer in order to be able to detect any potential differences in the tumors one step ahead of the protein level.
Material and methods

- 21 formalin-fixed, paraffin-embedded (FFPE) resection specimens from patients with metastasized urothelial bladder cancer.

- Immunohistochemistry for PD-L1 using different antibodies/assays (E1L3N - Cell Signaling and SP142 - Ventana).

- Scoring for immune and tumor cells (IC, TC) as well as for the CPS was performed.

- Consecutively RNA ISH was performed to detect PD-L1 RNA using the ViewRNA ISH Tissue 2-Plex Assay (Thermo Fisher) and the PD-L1 target specific probe set (VA1-14391-01).

- Slides were digitized and manually quantified for RNA signals.
RNA ISH demonstrates the presence of PD-L1 mRNA as red dots within tumor cell nuclei and cytoplasm (A, C).

The tissue without PD-L1 RNA specific probe set (C, D) respectively without the primary antibody (F) are negative.
Method – ViewRNA vs Immunohistochemistry

PD-L1 protein and mRNA expression in tonsil tissue is comparable.

Immunohistochemistry using PD-L1 specific antibodies / assays shows a positive reaction of comparable intensity.

PD-L1 mRNA expression (B) is detectable in the same tissue areas, in the crypts of tonsil (b1), in the germinal centers of follicles (b2) and in the interfollicular spaces using the PD-L1 RNA specific probe set.
Results

- In the IHC positive patient group, protein and RNA expression on tumor and immune cells show comparable results.

- In the IHC negative group, we found in a part of the samples PD-L1 RNA expression in various amount on tumor and/or cells.
Conclusion

- PD-L1 RNA and protein expression in tumor and immune cells matches in IHC positive cases.

- 58% of IHC negative cases show PD-L1 RNA expression in tumor cells (confirmed by RT-PCR).

- Our data confirm that the immunohistochemical negative tumors are a heterogeneous group and a part of them may respond to immune checkpoint blockade.

- The detection of PD-L1 RNA in situ may be an additional diagnostic tool that could improve the prediction of therapy response.

- A follow-up study regarding clinical data and therapeutic response is in progress to evaluate the predictive value of this approach.
Thank you for your attention!

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