Looking beyond the assay: Comparison of multiplex chromogenic and fluorescent immuno-histochemistry for standardized immune oncology profiling in non-small cell lung carcinoma patients

P39

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BACKGROUND

Given the heterogeneity of tumors and the variety of potential biomarkers in immune oncology, there is a need for quantitative standardized assays to reliably assess the immune status of a patient's tumor to be able to extract the true biological information across cohorts. Multiplexing is key for extraction of maximum amount of insights from a limited amount of tissue, including cellular interactions (multiplex chromogenic immunohistochemistry, mIHC) and cellular co-expression patterns (multiplex immunofluorescence, mIF). Here, the 2 different tissue-based approaches
mIHC and mIF have been compared using well validated assays. Independently of the technique used, assay reproducibility and standardized quantification of staining intensity are a prerequisite for obtaining consistent results. Using a cohort of non-small cell lung carcinoma (NSCLC) patients, we identified patterns of immune cell infiltration that were comparable, independent of the assay applied.

METHODS ASSAY

Formalin-fixed paraffin-embedded (FFPE) true consecutive slides from 7 NSCLC resections were stained with a multiplex chromogenic panel (including CD3, PD-L1, CD68, CD8, PD-1) at Mosaic Laboratories (1) and with the UltiMapper kits (I/O PD-L1 and I/O PD-1) from Ultivue (Figure 1). mIHC scans were acquired with an Aperio AT Turbo scanner (Leica), while mIF scans were acquired with a Zeiss Axio Scan.Z1 scanner (Zeiss) both as whole slide images. Scanned images were transferred to Definiens.

REFERENCES

1) Lisa M. Dauffenbach, et al. Characterization of inflammatory cell patterns and densities using multiplex immunohistochemistry immuno-oncology assays [abstract]. In: Proceedings of the AACR-NCI-EORTC International Conference: Molecular Targets and Cancer Therapeutics; 2017 Oct 26-30; Philadelphia, PA. Philadelphia (PA): AACR; Mol Cancer Ther 2018;17(1 Suppl): Abstract nr B069.
2) Lorenz Rognoni, et al. Automated quantification of whole-slide multispectral immunofluorescence images to identify spatial expression patterns in the lung cancer microenvironment. SITC Annual Meeting; 2018 Nov 7-11; Washington, DC. Poster nr P442.
3) Brieu, Nicolas, et al. (2019). Domain Adaptation-based Augmentation for Weakly Supervised Nuclei Detection.

METHODS IMAGE ANALYSIS

mIHC and mIF images were co-registered and annotated (Fig 1). Definiens custom algorithms for digital image analysis were applied (2,3). A Deep Learning (DL) model was used to detect the different cell populations (Fig 2). Tumor epithelium and stroma detection was based on a histology-driven DL approach for mIHC and on pan Cytokeratin for mIF (Fig 3). Densities of immune cell populations in different compartments (invasive margin *vs* tumor center and tumor epithelium *vs* tumor stroma) were measured as readouts from the two techniques mIHC and mIF, and the 2 datasets were compared using Spearman's rank correlation coeficient (Fig 4A). Additionally, the density of PD-L1 positive cells in the tumor epithelium was also compared across assays (Fig 4B).

RESULTS & CONCLUSIONS

Differences in mIHC and mIF based signal amplification lead to systematic differences for most markers

- Generally, more cells were detected on mIHC as compared to mIF
- For PD-L1, the mIF assay was more sensitive in the lower expression range

Even without optimization of the image analysis for optimal assay correlation, reasonable to good correlation can be achieved for most markers

- Adjustments of mIF detection thresholds might be sufficient for differentiation markers with narrow expression range (CD3, CD8)
- For markers with a broader distribution of expression levels (PD-L1, PD-1) a larger cohort of samples in a bridging study will be required.

This suggests that co-optimization of assays and/or bridging studies are required when performing cross-study comparisons with different multiplex assays. For more information please visit Poster 59!

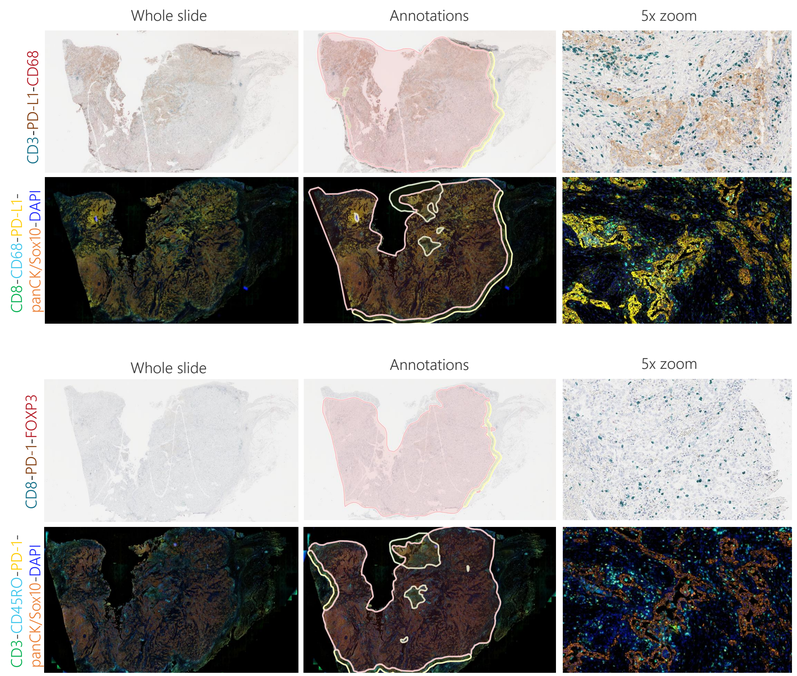

Figure 1: Consecutive slides from NSCLC resections stained with a multiplex chromogenic panel (top images) and with the UltiMapper PD-1 and PDL1 kits (bottom images). The annotations image highlights the areas that have been used for quantification: invasive margin in yellow, tumor core in red, exclusion areas in green. 5x zoom highlighting corregistration.

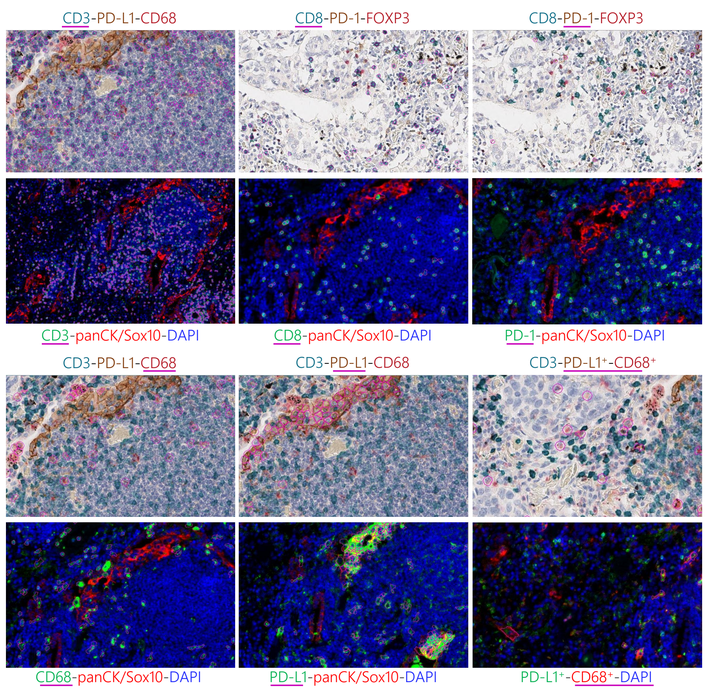

Figure 2: Artificial intelligence based cell detection (pink). 20x images highlighting the individual markers (respective color) and the detection in pink.

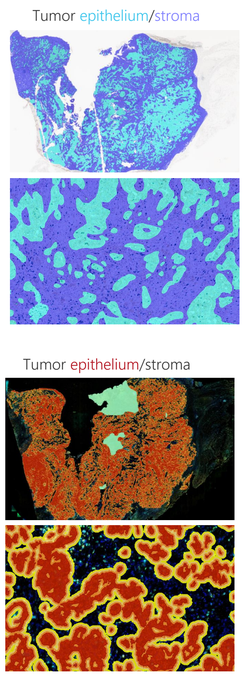

Figure 3: DL-based tumor epithelium & stroma detection. Top, mIHC epithelium in blue, stroma in purple. Bottom, mIF epithelium in red with yellow border, stroma in black.

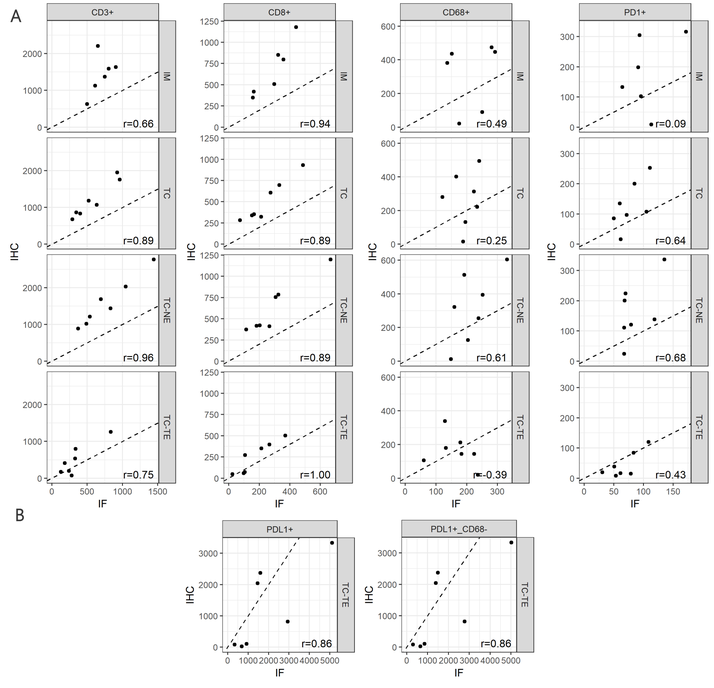

Figure 4: Spearman's rank correlation coefficients were calculated for: (A.) the different immune cell populations: CD3, CD8, CD68 and PD in the 4 compartments: invasive margin (IM), tumor center (TC), tumor epithelium (TC-TE) and tumor stroma (TC-NE); and (B.) for PD-L1 tumor cells in the tumor epithelium.