LB-275: Measuring concordance of CD8 and PD-L1 expression in Non-Small Cell Lung cancer between a novel multiplex immunofluorescence assay and a brightfield laboratory developed test

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Introduction

The quantitative assessment of Programmed death-ligand 1 (PD-L1) protein expression has been widely used in the clinic to predict response to immune checkpoint therapy in non-small cell lung cancer (NSCLC) patients. The conventional method of assessment is the manual quantification (scoring) of PD-L1 positive tumor cells by a board-certified pathologist on samples stained by immunohistochemistry (IHC). To improve the accuracy of the PD-L1 scoring, we assessed the use of digital image analysis tools to quantify the positive tumor cells and correlate the data with the conventional manual scoring method used in the clinical setting by both brightfield and multiplex immunofluorescent assays.

The PD-L1 expression in tumors is used either as a predictive or a prognostic biomarker and is often dependent on the presence of T cells in the tumor microenvironment. Here, we evaluate the digital analysis scoring of PD-L1+ tumor cells as well as CD8+ T cells in NSCLC samples stained by IHC and by the ImmunoVUE™ PD-L1 multiplex assay using InSituPlex® technology (ISP). We determine the percent of PD-L1+ tumor cells and of tumor infiltrating CD8+ T cells and show concordance between manual and digital analysis. Further, we also show positive correlation in PD-L1 and CD8 digital scoring by both ISP and brightfield assays.

Methods

Multiplex Immunofluorescence (mIF) was performed on thirteen NSCLC tissues using ImmunoVUE PD-L1 multiplex assay (consisting of PD-L1, CD8, CD68 and CK/Sox10 markers) based on Ultivue's InSituPlex® technology (Figure 1). Duplex PD-L1/CD8 IHC staining was performed on consecutive sections of the same thirteen tissues at reference laboratory (Massachusetts General Hospital). Manual scoring was performed by a board-certified pathologist in terms of percent of PD-L1 positive tumor cells showing membrane expression and percent of intra-tumoral CD8+ T cells.

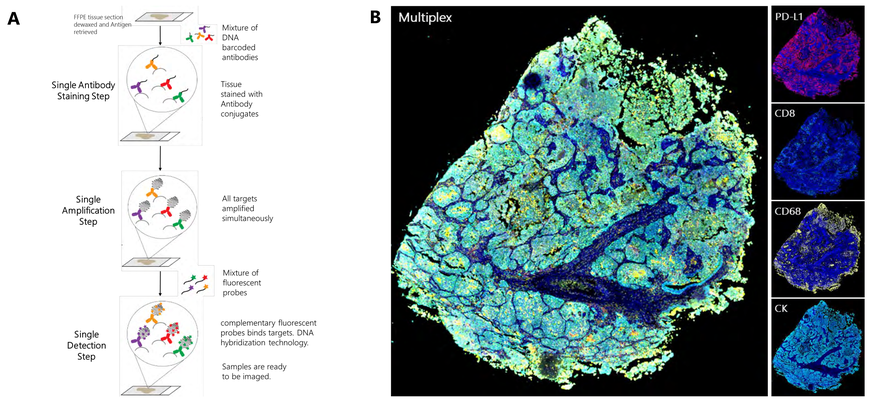

Figure 1. (A) InSituPlex and DNA exchange technology. (B) NSCLC image stained with the ImmunoVUE PD-L1 multiplex assay showing PD-L1 *(Cy5-Red)*, CD8 *(FITC-green)*, CD68 *(TRITC-Yellow)*, and CK *(Cy7-Cyan)*.

Digital image analysis workflow

Digital image analysis was performed using HALO®v3.0 software on multiplex ISP and IHC images previously used for manual scoring. For the digital analysis, the tissue was first annotated to select the area to be analyzed and then classified into tumor and non-tumor regions using random forest classification (Figure 2). Cell segmentation is performed using the appropriate modules to count the number of cells for each marker according to the classification.

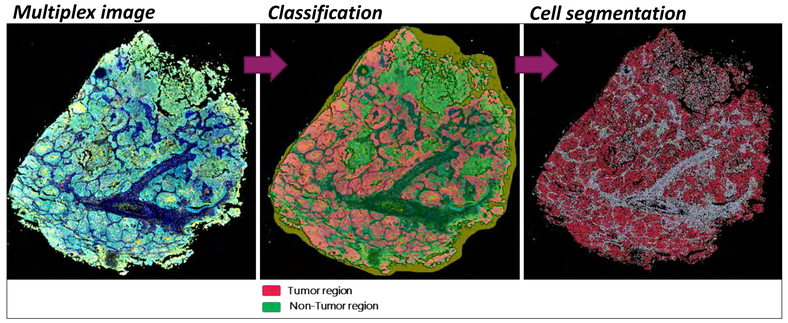

Figure 2. A schematic representation showing the image analysis workflow (L-R) which consists of annotating the tissue region for analysis, an overlay of the classification of the tissue into tumor and non-tumor regions, and cell segmentation according to marker and classification.

In this study, cell segmentation was performed using Cytonuclear algorithm (version 2.0) for brightfield images and HighPlex FL (version 3.1) for multiplex ISP images. The images were analyzed for total number of tumor cells, total number and percent of PD-L1+ tumor cells, and intra-tumoral CD8+ T cells. The total number and percentage obtained were compared to the manual PD-L1 and CD8 scoring.

Results

Digital analysis of the samples stained by IHC or by the ImmunoVUE PD-L1 multiplex assay showed no significant differences compared to the manual scoring in terms of total tumor cell count or the percent of PD-L1+ tumor cells and CD8+ T cells associated with the tumor. Figure 3 shows positive correlation and concordance between manual and digital analysis. The co-efficient of correlation was greater than 0.9 when comparing manual scoring to digital analysis in both ISP and IHC images.

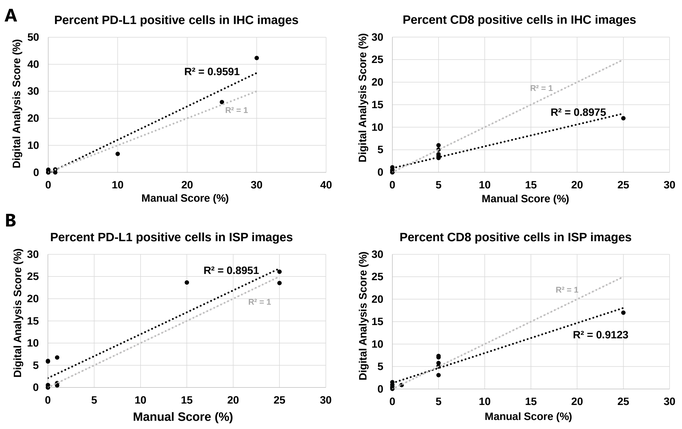

Figure 3. (A) Concordance between percent values of PD-L1+ and CD8+ cells obtained by manual scoring and digital analysis scoring in brightfield images. (B) Concordance between percent values of PD-L1+ and CD8+ cells obtained by manual scoring and digital analysis scoring in ISP images.

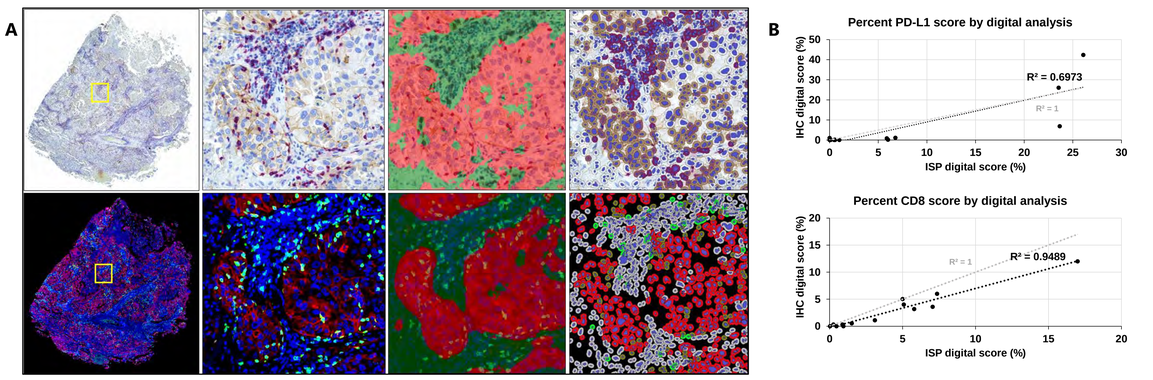

Figure 4. (A) Representative NSCLC image stained with IHC (top) with an enlarged area showing duplex PD-L1 (brown) and CD8 (Red) staining followed by classification into tumor (red) and non-tumor (green) regions and cell segmentation according to classification (L-R). ImmunoVUE PD-L1 assay staining (bottom) showing the same enlarged area as shown in IHC with only PD-L1 (Red) and CD8 (Green) staining followed by classification into tumor (red) and non-tumor (green) regions and cell segmentation according to the classification (L-R).
(B) Correlation between percent of PD-L1+ and CD8+ cells obtained by digital analysis scoring in IHC and ISP images.

Analysis methods or Assay	Coefficient of correlation	
	PD-L1	CD8
IHC Manual vs. Digital	0.98	0.95
ISP Manual vs. Digital	0.95	0.96
Digital analysis ISP vs. IHC	0.84	0.97

Digital analysis results obtained from IHC stained slide was concordant with an ImmunoVUE PD-L1 multiplex stained slide from the same sample showing correlation in terms of positive cell counts. The coefficient of correlation for PD-L1 between brightfield LDT and ISP was measured at 0.84 while that for CD8 was 0.97.

Conclusions

- This study shows that digital image analysis of PD-L1 positive tumor cells and CD8 positive T cells in non-small cell lung cancer (NSCLC) tissues provides concordant results with the conventional manual scoring method and can be used as an accurate method for PD-L1 scoring in clinical samples.
- The study also highlights the concordance in cell counts in NSCLC tissues with variable PD-L1 expression stained with either IHC or the ImmunoVUE PD-L1 multiplex assay showing the reliability of the PD-L1 multiplex assay and digital analysis.
- Lastly, the ImmunoVue PD-L1 multiplex assay appears to be concordant with an established LDT, as it shows positive correlation in cells counts compared to IHC method, making it an appropriate assay to use for PD-L1 and CD8 assessment in clinical samples. Also, the ImmunoVUE PD-L1 multiplex assay can be used to accurately identify multiple phenotypes in the same tissue.