Image Analysis and Manual Scoring of PD-L1 in Melanomas Using Physical and Virtual Double Staining

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INTRODUCTION

Programmed cell death-1 ligand 1 [PD-L1] is a transmembrane protein that binds to the inhibitory receptor programmed cell death 1 receptor [PD-1], causing a down regulation of the immune responses. PD-L1 is typically expressed in normal cells as macrophages but frequently also in tumor cells while PD-1 is typically expressed on cytotoxic T-cells and other immune cells. Tumor cells can upregulate PD-L1 expression and avoid being attacked by the body's immune system, making an interruption of the PD-1/PD-L1 interaction an attractive method for assisting the immune system in destroying tumor cells.

Assessment of PD-L1 expression remains under debate and is further complicated by multiple immunohistochemical (IHC) assays, different scoring criteria and the presence of tumor-infiltrating immune cells. Here we investigate the diagnostic potential of a PD-L1+SOX10 double IHC assay in melanomas compared to PD-L1 single IHC assays by manual assessment and digital image analysis [DIA] methods.

METHODS AND MATERIALS

28 melanomas assembled in a tissue microarray (TMA) were analyzed by IHC for PD-L1 and PD-L1+SOX10 and all melanoma samples were assessed by manual and DIA scoring.

Cells were classified as SOX10+/PD-L1+ based on presence of both markers, shared membrane between tumor cells and absence of surrounding SOX10-/PD-L1+ cells. For the single IHC assay, SOX10 was used to outline tumor regions using VirtualDoubleStaining™ and the level of PD-L1 expression was subsequently evaluated in tumor cells. Negative reagent controls were performed to exclude a false positive PD-L1 result due to pigment.

Tumor Proportion Scores [TPS] were calculated as:

$$TPS = \frac{\# \text{ of PD-L1 positive tumor cells}}{\# \text{ of total tumor cells}}$$

From the scores, each sample was grouped into <1%, 1–5% and >5% and compared to the manual assessment of PD-L1 stained samples. All TPS were corrected for pigment by subtracting the TPS of negative TAMs before grouping.

RESULTS

The distribution of classifications for each method is shown in Figure 4 and the TPS of four different methods shown for each TMA in Figure 5.

Comparing manual scoring of PD-L1 and PD-L1+SOX10, there were 23 of 28 samples scored below 1% for the double stain, resulting in 11 cases where the single stained samples were scored higher.

PD-L1+SOX10 DIA scored 9 samples higher and 3 samples lower compared to manual PD-L1 assessment, while PD-L1 DIA scored 21 samples higher. The average TPS score of the single, double and stained stained samples were 2.3, 29% and 10.2%, respectively.

CONCLUSION

Differences in PD-L1/SOX10 and PD-L1 scoring indicates that PD-L1 expression is diminished in double stained samples. However, the interpretation of tumor-infiltrating immune cells might affect the assessment and was seen in the absence of SOX10 tumor identification. DIA scored PD-L1/1SOX10 higher compared to manual PD-L1/1SOX10 scoring, mainly in samples with low-positive and cytoplasmic staining. DIA of PD-L1/1SOX10 matches manual PD-L1 scoring more closely than DIA of PD-L1 and maybe advantageous for disregarding tumor-infiltrating immune cells.