Quantification of PD-L1 Expression on Tumor Cells in Non-Small Cell Lung Cancer
Using Non-Enzymatic Tissue Dissociation and Flow Cytometry (OncoTect iO™ Lung)

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OBJECTIVE

Tumors use many mechanisms to evade the immune system, often manipulating pathways to evade cell death. The PD-L1/PD-1 pathway in particular, has been shown to be a promising target for immuno-oncology drug development. PD-L1/PD-1 therapy has been shown to be effective in patients with non-small cell lung cancer (NSCLC), regardless of their PD-L1 expression profile by immunohistochemistry (IHC). This creates a challenge in determining potential responders prior to treatment, especially when immuno-oncology drugs carry a hefty price tag. The objective of this study was to develop a truly quantitative technology for PD-L1 expression in NSCLC. In addition, we also present a non-enzymatic technology that creates a tumor cell suspension from fresh tumor tissue so that either fine needle aspiration or fresh tissue can be used in this assay.

METHODS

NSCLC biopsy tissues of >150 mg were obtained for this study. 4 mm punches were taken from each tumor tissue. Non-enzymatic tissue homogenization (incellPREP; IncellDx, Menlo Park, CA) was performed to create a single cell suspension from the tissue punches. Cells were fixed and permeabilized, labeled with antibodies directed against CD45 and PD-L1 (BioLegend 29E.2A3), then stained with DAPI to identify intact, single cells, and to analyze cell cycle. Antibodies to CD3 and CD8 were added in later testing to separate immune cell subsets.

RESULTS

10 of 12 lung tumor samples were concordant for PD-L1 detection while 2 were discordant; one positive by flow and negative by IHC and one negative by flow and positive by IHC. PD-L1 expression by flow cytometry varied widely (1.2% to 89.4%) even in the positive concordant cases (Table 1).

In addition, samples with overall high PD-L1 expression, showed increased expression in the aneuploid tumor component. Table 2 contains NSCLC patient sample data with antibodies to CD3 and CD8 included with PD-L1 (Abcam 28-8), CD45, and DAPI. The addition of CD3 and CD8 allows for the identification of Cytotoxic T-cells and T-Helper cells and their PD-L1 expression. PD-L1 expression is represented 2 ways, as a percent expression of the population and as receptors of PD-L1 using MESF bead standards (Bangs Laboratories). Here a range of expression can be seen across NSCLC tumor samples.

CONCLUSION

PD-L1 quantification on tumor and immune cells in NSCLC may allow for better prediction of response to PD-1/PD-L1 pathway inhibitors.