Background and Results

Background: Non-small cell lung cancer (NSCLC) accounts for approximately 84-85% of all lung cancer cases, and is characterized by a poor 5-year survival and a low 5-year survival. Treatment targeting the immune checkpoint inhibitory pathway PD-L1/PDL1 has been found to be effective against NSCLC with non-small cell type, but with only 30-40% of patients showing a positive response. There is an urgent need for additional immunotherapeutic options for this group of patients. Lymphocyte Activation Gene-3 (LAG-3) has been recognized as a new checkpoint pathway in the inhibition of T lymphocytes, with its expression on activated T cells, and is often found in co-expression with PD-1 leading to immune exhaustion and tumor growth. Co-blockade of the LAG-3 and PD-1 pathway has been shown to synergize to improve T cytotoxic cell responses, and several LAG-3 modulating agents (BMS 986016, LAG525, MK-4280, REGN3767) have entered the clinic where they are currently being tested alone or in combination with PD-L1/PD-1 pathways inhibition.

Methods: In order to perform a comprehensive immunoprofiling of NSCLC tumors we used Multiplexing™, an immunofluorescence (IF) multiplexing assay that utilizes a pair of directly conjugated Cyanine dye-labeled (Cy3, Cy5) antibodies per round of staining. Using a 16-marker panel we have assessed the proportion of 6 subtypes, i.e., cell subsets, tumor-associated macrophages (TAMs), as well as the expression of PD-1, LAG-3, and TIM-3 in 10 samples from patients with NSCLC.

Results: LAG-3 and PD-1 found to be expressed mainly on T cells, with the proportion of T cytotoxic cells relative to T helper cells increasing inside the tumor area. When analyzing the proportion of T cytotoxic cells infiltrating into the tumor area, we obtained an apparent synergism between LAG-3 and PD-1 in expressing LAG-3 and PD-1 in the Peritumoral area, suggesting the therapeutic benefit of dual checkpoint blockade of LAG-3 and PD-1 in NSCLC.

Conclusion

In this study, utilizing Multiplexing™ technology, a platform delivered exclusively by NeoGenomics laboratories, protein expression in 19 NSCLC patients were analyzed for analysis of PD-1 and LAG-3 expression in the tumor microenvironment.

• Both PD-1 and LAG-3 were found primarily expressed on T cells. While LAG-3 was found mainly on CTLs in both the stroma and inside the tumor area, PD-1 was found mainly on T helper cells in the stroma but on CTLs inside the tumor area.

• When analyzing the tumor infiltration mask generated as described in Figure 3, the density of LAG-3+ cells was found to be highest in the stroma, while LAG-3+ cells were also found inside the tumor area. Overall, LAG-3+ cells were more abundant in the stroma than in the tumor area.

PD-1 & LAG-3 Synergize in Increasing Tumor-Infiltration of T Cytotoxic Cells

PD-1 and LAG-3 Synergize to Drive Tumor-infiltration of T Cytotoxic Cells in NSCLC Tumors

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