Background and Results

Background: Non-small cell lung cancer (NSCLC) accounts for approximately 80-85% of all lung cancer cases, and is characterized by a poor response to chemotherapy and a low survival rate. NSCLC is a very heterogeneous disease with the two most common types being adenocarcinoma (ADCA) and squamous cell carcinoma (SCC) accounting for about 30% of all NSCLC cases, and squamous cell carcinoma (SCC) for about 20% of all cases. Treatment targeting the immune checkpoint inhibitor pathway PD-1/PD-L1 has been found to be effective against NSCLC with manageable side effects, but with only 20-30% of patients showing a positive response there is an urgent need for additional immunotherapy options for this group of patients.

Methods: We used MultiOmyx™, an immunofluorescence (IF) multiplexing assay that utilize a panel of directly conjugated Cyanine dye-labeled (Cy3, Cy5) antibodies per round of staining. Using a 16-marker panel we have analyzed the proportion of B cells, T cell subtypes, myeloid type TAMs in SCC versus ADCA tumors. LAG-3 was found to be expressed mainly on T cytotoxic cells in both subtypes, but the overall density of LAG-3 was 39% higher in SCC. TIM-3, a primarily found expressed on T cells had a 15% higher density in SCC, while the density of PD-1 was 33% lower in SCC compared. When analyzing the proportion of T cytotoxic tumor-infiltration, we observed an apparent synergy between LAG-3 and PD-1 co-expression in both ADCA and SCC, suggesting a therapeutic benefit of dual checkpoint blockade of LAG-3 and PD-1 in NSCLC.

Results: We observed a higher density of the immunosuppressive cell types Tregs and M2 type TAMs in SCC versus ADCA tumors. LAG-3 was found to be expressed mainly on T cytotoxic cells in both subtypes, but the overall density of LAG-3 was 39% higher in SCC. TIM-3, a primarily found expressed on T cells had a 15% higher density in SCC, while the density of PD-1 was 33% lower in SCC compared. When analyzing the proportion of T cytotoxic tumor-infiltration, we observed an apparent synergy between LAG-3 and PD-1 co-expression in both ADCA and SCC, suggesting a therapeutic benefit of dual checkpoint blockade of LAG-3 and PD-1 in NSCLC.

Conclusion

In this study, utilizing MultiOmyx™ technology, a platform offered exclusively by NovoGenomics Laboratories, protein-expression in 10 patients with lung cancer were analyzed for a comparison of the NSCLC subtypes adenocarcinoma (ADCA) and squamous cell carcinoma (SCC). By analyzing a panel of 36 antibody markers we quantified the number of T cells, B cells, TAMs, and M2 type TAMs and observed an increase in the proportion of the immunosuppressive cell types: T regulatory cells, and M2 TAMs in SCC versus ADCA. Additionally, SCC samples had a higher density of both LAG-3 and TIM-3. In both subtypes LAG-3 was found primarily expressed on T cytotoxic cells, while TIM-3 was found primarily on TAMs, followed by an equal distribution on helper and cytotoxic T cells. When analyzing T cytotoxic tumor-infiltration we found that in both ADCA and SCC, cells co-expressing LAG-3 and PD-1 infiltrated the tumors to a much more than cells expressing LAG-3 or PD-1 alone. This apparent synergy between LAG-3 and PD-1 was not observed for TIM-3 and PD-1.

Using a Multiplexed Immunofluorescence Approach to Compare Immune Cell Populations in Subtypes on Non-Small Cell Lung Cancer

Anna Juncker-Jensen • Vivek Reddy • Erin Farnell • Máté Nagy • Judy Xue • Eric Leones • Flora Safaie • Kathy Pham • Joerette William

Clinical Laboratories, Alacare, Inc.