TIGIT (T cell immunoreceptor with Ig and ITIM domains) is a recently identified immune receptor that is expressed on T cells, natural killer (NK) cells and NKT cells. TIGIT has emerged as an important coinhibitory receptor. TIGIT expression on CD8+ tumor infiltrating lymphocytes (TILs) has been shown to be upregulated in solid cancers such as melanoma, colon cancer, and NSCLC and has been associated with a dysplastic phenotype in TILs. TIGIT can also be activated in a subset of Regulatory T cells (Tregs) and may be critical in driving CD8+ T cell dysfunction. A second mechanism in which TIGIT inhibits immunosurveillance is through both competition and direct inhibition of CD226, impairing its ability to activate immunosurveillance. TIGIT has also been reported to inhibit T cell responses indirectly by triggering CD155 expression on dendritic cells (DCs), thereby preventing DC maturation. These findings render the TIGIT pathway as an attractive candidate for cancer immunotherapy.

In this study, we seek to use MultiOmyx hyperplexed immunofluorescence (IF) assay to exploit this new pathway and characterize the TIGIT expression in a total of 17 melanomas and NSCLC samples. The cancer FFPE slides were stained with a 12-color marker panel including CD20, CD3, CD4, CD8, CD10, CD11c, CD16, CD206, CD38, CD45, FoxP3, and SOX10. This panel enabled the detection of TIGIT, CD206 and CD10 expression in the melanoma and NSCLC samples. The TIGIT expression was further characterized on different T cells including CD4+ helper T cells, CD8+ cytotoxic T cells, CD56+ NK cells. Using the MultiOmyx proprietary algorithm, we can quantify different subtypes of TIGIT expressing cells and measured the distance of different TIGIT expressing cells to the tumor. Increased expression of TIGIT and PD-1 has been demonstrated in NSCLC and melanoma. Moreover, TIGIT+ Tregs have been reported to upregulate TIM-3 expression in mouse tumor models. This study evaluated the expression of TIGIT in conjunction with other coinhibitory receptors such as PD-L1, LAG-3 and TIM-3. The population of TILs that co-express TIGIT/PD-L1, TIGIT/LAG-3 and TIGIT/TIM-3 were quantified and analyzed.

Leveraging TIGIT in combination with other immune therapy may achieve more robust clinical outcomes. There are currently multiple phase I clinical trials using TIGIT monotherapy in combination with anti-PD-1/L1 or antibodies in solid tumors. Our data can help provide more insight into how TIGIT modulates antitumor immunity in melanomas and NSCLC samples. The findings in this study can also be used to understand the synergistic effects between TIGIT and other coinhibitory receptors and help identify the additional opportunity for combination immunotherapy using checkpoint inhibitors.

**Overview of Assay Workflow**

1. Acquire Background
2. Stain Slide
3. Acquire immunofluorescence

**Expression of TIGIT in Different Subtypes of Lymphocytes**

**Cell Classification and Spatial Analysis of TIGIT Expressing T cells**

- A. Heat map of MultiOmyx TIGIT expressing cell classification results in NSCLC and melanoma. The results are given as densities.
- B. Nearest neighbor analysis to calculate the average of the distance of the 5 nearest neighbors from any given phenotype.
- C. Spatial correlations of TIGIT expressing T cells to tumor cells in NSCLC samples.
- D. Spatial correlations of TIGIT expressing T cells to tumor cells in melanoma samples.

**Co-expression Analysis of TIGIT with Immune Checkpoint Inhibitors**

**Figure 3.** Analytics results of TIGIT expressing T cells in NSCLC and melanoma specimens. A. Heat map of MultiOmyx TIGIT expressing cell classification results in NSCLC and melanoma. The results are given as densities. B. Nearest neighbor analysis to calculate the average of the distance of the 5 nearest neighbors from any given phenotype. C. Spatial correlations of TIGIT expressing T cells to tumor cells in NSCLC samples. D. Spatial correlations of TIGIT expressing T cells to tumor cells in melanoma samples.

**Figure 4.** Characterization of TIGIT expression with immune checkpoint inhibitors in NSCLC and Melanoma specimens. Representative color overlaid images showing co-expression of TIGIT with PD-L1, LAG-3 and TIM-3 in NSCLC and melanoma (Fig. A) and melanoma (Fig. B). Magenta arrows indicate examples of cells co-expressing TIGIT and PD-L1, yellow arrows indicate examples of cells co-expressing TIGIT and LAG-3, and red arrows indicate examples of cells co-expressing TIGIT, PD-L1, and LAG-3. C. Heat map of MultiOmyx TIGIT expressing T cells classification results in NSCLC and melanoma. D. Heat map of MultiOmyx TIGIT expressing cell classification results in melanoma. E. Heat map of MultiOmyx TIGIT expressing cell classification results in NSCLC. F. Heat map of MultiOmyx TIGIT expressing cell classification results in NSCLC.