Introduction

Background: Head and neck squamous cell carcinoma (HNSCC) is a cancer with the ability to modulate the immune system to evade detection. It is the sixth most frequently diagnosed cancer with 350,000 new cases and 320,000 lives lost worldwide each year. New treatments for HNSCC are urgently needed as patients continue to experience a high mortality rate and low response to surgery and chemotherapeutic treatments. Part of the reason why HNSCC is difficult to treat is its capacity to upregulate the expression of immune checkpoint molecules (TIGIT, PD-L1, LAG-3). These molecules are involved in immune responses to cancer and are expressed in a variety of markers essential in cancer immunology. Sequential tissue sections were stained in two panels, an exhaust T cell panel comprised of TIGIT, PD-1, LAG-3, CD4 and CD8 and a T cell panel including CD3, FOXP3, CD8, pan-cytokeratin, CD4 and CD8.

Methods: In this study, we sought to establish a robust report of immune cells in the tissue of patients with HNSCC. Using Vectra Polaris multiplex immunofluorescence assays, we studied T cell expression and T cell exhaustion in HNSCC patient tissues using a total of 7 markers essential in cancer immunology. Sequential tissue sections were stained in two panels, an exhaust T cell panel comprised of TIGIT, PD-1, LAG-3, CD4 and CD8 and a T cell panel including CD3, FOXP3, CD8, pan-cytokeratin, CD4 and CD8.

Results: Multiplexing if staining revealed a HNSCC histologic landscape characteristic of immune suppression in this study. The data demonstrated abundant T cells in the tissue microenvironment of HNSCC samples. Using India Halo algorithms, we quantitated T helper cells (CD3+CD4+), T cytotoxic cells (CD3+CD8+), T regulatory cells (CD3+CD4+FoxP3+), and different subtypes of exhausted T cells, within the tumor and thestromal regions.

Conclusions: Currently A11+1, a fully humanized immunoglobulin G1 monoclonal antibody targeting human TIGIT is in phase I trials in patients with HNSCC and I1217, an anti-PD-1 monoclonal antibody in combination with anti-CT1-1 monoclonal antibody. Tumour IHC is in a phase I/II clinical trial in patients with advanced solid tumors. The Vectra Polaris’ imaging reported in this study identifies T cell composition in the tumor microenvironment of patients facing high mortality and the findings in this study can be used to identify the additional opportunities for combination immunotherapy.

Assay Development & Panel Specifications

• Automated staining workflow
• A robust report of immune cells in the tissue of patients with HNSCC
• Sequential tissue sections were stained in two panels, an exhaust T cell panel comprised of TIGIT, PD-1, LAG-3, CD4 and CD8 and a T cell panel including CD3, FOXP3, CD8, pan-cytokeratin, CD4 and CD8.

Characterization of Immune Suppressive Cells in HNSCC

• HNSCC samples in this study.
• Exhausted T cell panel comprised of TIGIT, PD-1, LAG-3, CD8, CD4 and CD8 markers. B. Sequential order of HNSCC tissue in I and bare used with exhausted T cell panel including CD3, FOXP3, PD-1, CD4 and CD8. Fluctuations in T cell analysis were used for comparison in this study.

Key Findings

• Two panels were used in this study to characterize the immune landscape of FFPE samples from HNSCC patients.
• Multiplexing if staining revealed a HNSCC histologic landscape characteristic of immune suppression in this study. Overall, helper T cells were more prevalent than cytotoxic T cells in this study.
• Different subtypes of exhausted T cells were observed in the study. Co-expression of PD-1+TIGIT+, PD-1+LAG-3, TIGIT+LAG-3+ and PD-1+TIGIT+LAG-3+ in T cells were also present in the TME of HNSCC samples in this study.
• Halo algorithms were used to study the spatial correlation of T cells with respect to tumor cells.

Table 1. Panel Specification

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<th>Panel</th>
<th>CD3</th>
<th>FOXP3</th>
<th>PD-1</th>
<th>CD4</th>
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Table 2. Phenotyping of T cells and exhausted T cells

<table>
<thead>
<tr>
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<th>CD3</th>
<th>CD20</th>
<th>CD4</th>
<th>CD8</th>
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<td>T helper cells</td>
<td>100</td>
<td>100</td>
<td>100</td>
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<tr>
<td>T regulatory cells</td>
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<td>100</td>
<td>100</td>
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<td>T cytotoxic cells</td>
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Figure 1: HNSCC Analysis. A. Demonstration of robust workflow. B. Panel staining. C. Automated algorithms and antibodies were applied to 4 in 1 slides of tissue stained followed by automated analysis and secondary IFAS. D. Most important module of counting with another marker and IFAS. E. Exonunneous antibody antibody algorithm generates positive detections.

Figure 2: HNSCC Image Analysis. A. T cell. B. Exhausted T cell. C. Helper T cell. D. PD-1+TIGIT+LAG-3+ in T cells were also present in the TME of HNSCC samples in this study. E. Exhausted T cell panel regional scoring was used to grade the A11+ exhaust T cell panel image. F. Exhausted T cell panel regional scoring was used to grade the A11+ exhaust T cell panel image. There is no indication of tissue microenvironment.

Figure 3: Characterization of immune suppressive microenvironent in HNSCC. A. Exhausted T cell panel. B. Exhausted T cell panel regional scoring was used to grade the A11+ exhaust T cell panel image. C. Helper T cell panel. D. Exhausted T cell panel. E. Exhausted T cell panel regional scoring was used to grade the A11+ exhaust T cell panel image. F. Exhausted T cell panel regional scoring was used to grade the A11+ exhaust T cell panel image. G. Exhausted T cell panel regional scoring was used to grade the A11+ exhaust T cell panel image. H. Exhausted T cell panel regional scoring was used to grade the A11+ exhaust T cell panel image.

Figure 4: Key Findings. A. Two panels were used in this study to characterize the immune landscape of FFPE samples from HNSCC patients. B. Multiplexing if staining revealed a HNSCC histologic landscape characteristic of immune suppression in this study. C. Helper T cells were more prevalent than cytotoxic T cells in this study. D. Different subtypes of exhausted T cells were observed in the study. Co-expression of PD-1+TIGIT+, PD-1+LAG-3, TIGIT+LAG-3+ and PD-1+TIGIT+LAG-3+ in T cells were also present in the TME of HNSCC samples in this study. E. Halo algorithms were used to study the spatial correlation of T cells with respect to tumor cells.