Differential Expression (DE) of genes in cells expressing PIK3C isoforms was performed. We used the COLD method implemented in Seurat to run DE analysis between the given group of cells (e.g., PIK3C expressing vs. the rest of the cells of the same type).

### 1: PIK3CD and PIK3CG Expression in Tumors

- Levels of PIK3C isoforms were determined in head and neck squamous cell carcinomas (HNSCC) across nearly all of 18 primary HNSCC tumors.
- PIK3CD expression is higher than PIK3CG across nearly all of HNSCC tumors compared to normal tissue.

### 2: PIK3C Gene Expression in Cancer Cells & DE Analysis

- Analysis of PIK3C gene expression in head and neck squamous cell carcinoma (HNSCC) across 18 primary tumors revealed that:
  - PIK3CA, PIK3CB, and PIK3CD are expressed in a significant number of malignant cells in HNSCC.
  - PIK3CD expression is significantly higher compared to PIK3CG across nearly all tumors.

### 3: Gene Expression in Tumors

- Cancer cells were identified by gene expression signature in PIK3C gene expression.
- PIK3CD median expression is higher than PIK3CG across nearly all of HNSCC tumors.
- PIK3CD gene expression is found in both head and neck cancer and other tumor types.
- PIK3CG expression is found in both head and neck cancer and other tumor types.

### Figures

- Figure 1: Hierarchical clustering of PIK3 isoforms in HNSCC Cancer Cells
- Figure 2: Gene expression thresholds for PIK3CD, PIK3CG, and PIK3CB (A 0.3 log TPM threshold (dashed line) was applied for PIK3CA and cancer cells, and was marked by red line).
- Figure 3: PIK3 expression in Cancer Cells & DE Analysis
- Figure 4: Expression in tumors
- Figure 5: Gene expression thresholds for PIK3CD, PIK3CG, PIK3CB and PIK3CA (A 0.3 log TPM threshold (dashed line) was applied for PIK3CA and cancer cells, and was marked by red line).
- Figure 6: Expression in tumors

### Tables

<table>
<thead>
<tr>
<th>Table 1: Frequency of PIK3C gene expressing Cancer Cells in HNSCC tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PIK3CA</strong></td>
</tr>
<tr>
<td>HNSCC</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2: Top Differentially Expressed Genes for PIK3C Expression in HNSCC Cancer Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PIK3CD</strong></td>
</tr>
<tr>
<td>4.43E-12</td>
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</table>

### Acknowledgements

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### Disclosures

No significant other relationships were reported with respect to the study and any of the authors or their institutions have any conflicts of interest to disclose.

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**Figure 7: Hierarchical clustering of PIK3 isoforms in HNSCC TME Cells**

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**Table 4: Percentage of Cells Expressing PIK3 Isoforms in HNSCC Tumors**

<table>
<thead>
<tr>
<th><strong>PIK3 Isoform</strong></th>
<th><strong>HNSCC</strong></th>
<th><strong>Colon Adenocarcinoma</strong></th>
<th><strong>Lung Adenocarcinoma</strong></th>
<th><strong>melanoma</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>PIK3CA</td>
<td>60%</td>
<td>50%</td>
<td>40%</td>
<td>30%</td>
</tr>
<tr>
<td>PIK3CB</td>
<td>24%</td>
<td>28%</td>
<td>22%</td>
<td>20%</td>
</tr>
<tr>
<td>PIK3CD</td>
<td>16%</td>
<td>22%</td>
<td>20%</td>
<td>10%</td>
</tr>
</tbody>
</table>