Intensification: Baseline signatures have so far failed to accurately predict response to immune checkpoint inhibitors. A model that mirrors the cellular response to tumor immune microenvironment (TME) upon treatment with Nivolumab (Nivo)+Ipilimumab (Ipili) may help us stratify responders. Tumor histocultures capture the complex interactions between tumor microenvironment components effectively. They remain the immune model choice with spatial context. The Farcast™ TiME histoculture platform provides a living microenvironment with near native fidelity to elucidate treatment-responder patterns that closely mimic patient responses.

Methodology: Patient tissue samples were surgically resected Head and Neck Squamous Cell Carcinoma (HNSCC) patient samples were collected from consented patients. A matched blood sample from the patient was also collected.

Histoculture building: The tumor sample was processed to generate thin explants without enzymatic digestion to retain the tumor microenvironment. The tumor explants were cultured with media and autologous plasma. The explants were treated with immune checkpoint inhibitor Nivolumab (anti-PD1)+Ipilimumab (anti-CTLA4) for 7 days. At day 7, the tumor explants were fixed with 4% formaldehyde and were stained with immune markers antibodies and intracellular antibodies. Data was acquired in BD LSR Fortessa using appropriate compensation controls and analyzed using FlowJo software.

Flowcytometry: The negative control was set as unstained and isotype control (anti-CD19, IgG4 in 1 µg/ml). The samples were analyzed using Leica automated multi-fluor system and Leica TME automated staining system respectively. Scoring was performed by certified pathologists. From H&E-stained slides, tumor content, immune content and baseline immune rvel (Tumor immune profile prior to tumor evaluation) was evaluated. Cleaved caspase 3 staining was evaluated in the tumor compartments.

Farcast™ TiME histoculture platform:

Figure 1: Schematic representation of the Farcast™ TiME histoculture platform and multi-dimensional assay for various immune responses. Immunohistochemistry-stained evaluation of PD1 marking observed upon Nivolumab treatment (Bottom).

Figure 2: (A) Tumor content evaluated using CoI stained images post treatment. (B) Low T cell activity assessed by immune-gene expression (PD1) compared to (C) Tumor treated with Nivolumab+Ipilimumab (Tumor treated with Nivolumab+Ipilimumab). (D) Median of T cell activity assessed by immune-gene expression (IL-2, IFNgamma, TNFa) compared to (E) Negative control (immune-gene expression (IL-2, IFNgamma, TNFa) compared to Negative control). (F) Representative data of expression analysis performed, through flowcytometry treatment within the Tumor segment in the two samples. Optimum T cell (CTLA4/Treg markers are shown as T cell activity and tumor proliferation marker are shown as Tumor markers).

Figure 3: (A) Multidirectional assay results using stimulated response rates in four samples (B) QuantiResp based extraction and segmentation strategy used for multiple HPC spatial data analysis. (C) Total CD3+ cell count and their spatial distribution characteristic is shown graphically for each sample. (D) Expression ratio images from the control (IgG4) and treated (Nivolumab) arms are shown.

Table 1: Analysis of immune checkpoint inhibitor.