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An ex vivo 3D tumoroid model of fresh patient tissue (3D-EXplore) to assess transcriptional and compositional changes of the Immune landscape in intact tumor microenvironment using Single-Cell Proteogenomics

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Background

- **Molecular changes underlying immune cell signaling in response to new therapy development are crucial to validate the clinical efficacy of immunotherapeutics. There are several preclinical tools including patient derived cell lines, organoid and xenograft (PDX) models to assess the efficacy of drugs. However, it is important to recapitulate the complexity of human malignancy and immune contexture within the tumor microenvironment.**
- **Here we report, an ex vivo platform (3D-EXplore) using fresh patient tumor samples with intact stromal and immune cell components to assess treatment-mediated changes in molecular and transcriptional profiles of tumor resident immune cells using a Cellular Indexing of Transcriptomes and Epitopes by sequencing (CITE-seq) platform.**

Materials & Methods

- **Tumor tissue procurement:** All tumor samples were obtained with patient consent and relevant IRB approval. All 3D *ex vivo* studies were performed with fresh colorectal carcinoma (CRC) tumor tissue.
- **3D-EXplore platform:** Unpropagated 3D tumoroids measuring 150 µm in size with intact tumor immune microenvironment were prepared from fresh tumor samples of colon and lung tissues using proprietary technology developed at Nilogen Oncosystems. No enzymatic digestion, propagation or reassembly was used during the preparation of the tumoroids. Hundreds of tumoroids originated from different parts of each patient's tumor sample were pooled to represent the tumor heterogeneity and treated ex vivo with or without immunostimulatory agents for 48h.
- **scRNAseq with CITEseq:** Multi-modal CITE-seq profiling using the 10X Genomics platform to interrogate cellular responses to ex vivo treatment. As a surrogate to determine treatment-induced immune cell activation of resident tumor infiltrating lymphocytes culture supernatants were collected for multiplex analysis of cytokine release in media .

Results

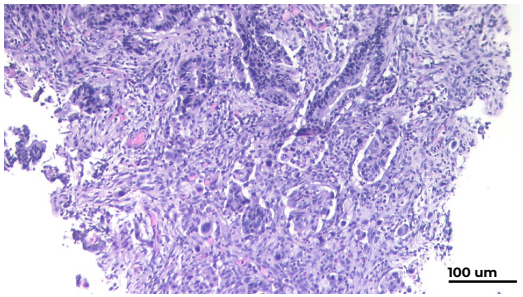

Figure 1. H&E depicting the diverse microenvironment within the CRC tumor tissue. Scale Bar = 100µm

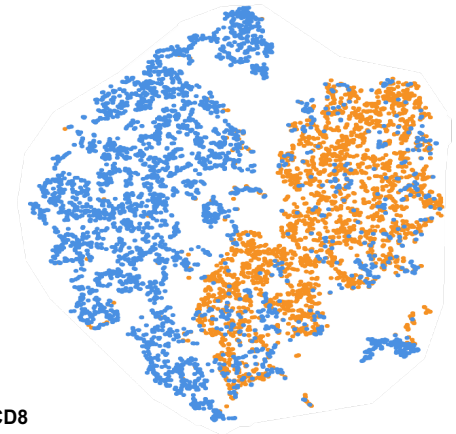

Figure 2. Multi-modal CITE-seq profiling (Above). 10X Genomics scRNAseq: t-SNE clustering to identify populations of CD45+ cells sorted from the untreated control group (Orange) and the STING-agonist treated group (Blue). Treatment with STING agonists results in a large shift in gene expression at the single-cell level as indicated by the altered clustering of cells.

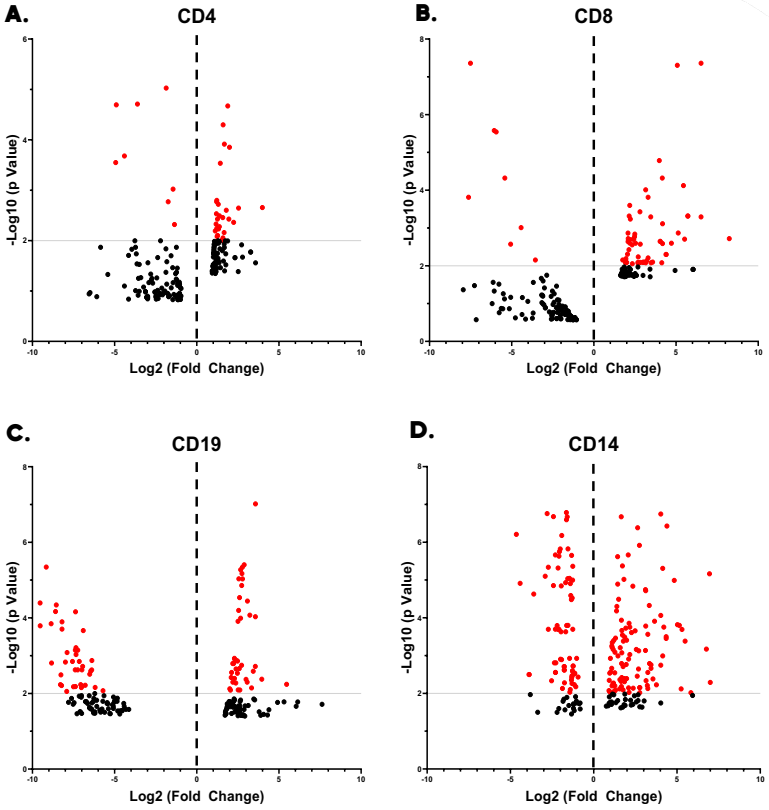

Figure 3. Volcano Plots from populations identified by CITEseq (Left). Volcano plots showing expression of the top 100 increased and decreased genes within immune cell populations identified using CITEseq feature barcoding coupled with gene expression in **(A)** CD4+ T cells, **(B)** CD8+ T cells, **(C)** CD19+ B cells, and **(D)** CD14+ myeloid cells. Red dots indicate significance (p<0.01)

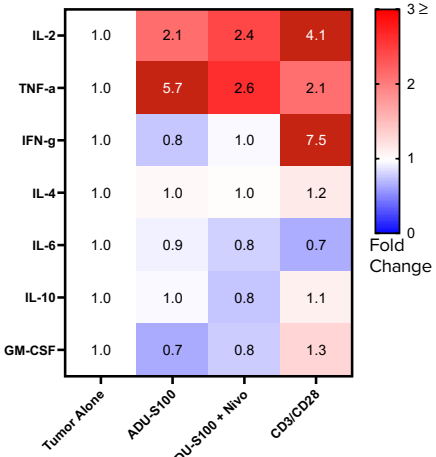

Figure 4. Cytokine production. Multiplex cytokine analysis was used to detect treatment mediated changes in cytokine production as a surrogate of immune cell activation within tumoroids treated with immunostimulatory STING agonists. Data is displayed as the fold change of treatment groups from Tumor Alone negative control.

Summary & Conclusions

- **Our data shows that we can detect the activation of tumor resident immune cells treated with immunostimulatory agents.**
- **The stimulation with STING agonists resulted in alterations in gene expression observed at the single-cell level and within specific immune cell populations using CITEseq coupled with 10x Genomics scRNAseq technology.**
- **Activation of the cGAS-STING pathway increased T cell activation and pro-inflammatory cytokine production.**
- **Nilogen's 3D-tumoroid platform provides a novel model to analyze treatment-mediated changes within the complex tumor microenvironment.**

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