CHARACTERIZATION OF THE SPATIAL AND TEMPORAL DISTRIBUTION OF TUMOR RESIDENT IMMUNE CELL POPULATIONS IN A 3DEXPLORE HUMAN TUMOROID MODEL AND ASSESSMENT OF RESPONSE TO IMMUNOTHERAPEUTICS EX VIVO

Seth Curlin*, Sharon Camacho, Angie Rivera, Jared Ehrhart, Soner Altiok. Nilogen Oncosystems, Tampa, FL, USA

Background Tumor cells cause significant chemical, cellular and physical alterations to surrounding tissue. The resulting tumor microenvironment (TME) includes a diverse population of immune and stromal cells contributing to tumor development and response to immunotherapeutic drugs. The 3D-EXplore ex vivo tumoroid platform enables high-throughput drug discovery within human tumor samples with intact TME for improved clinical translation. The work herein provides an in-depth assessment of the heterogeneous TME for multiple cellular populations including immune, stromal and tumor cells, as well as non-cellular components of the extracellular matrix (ECM) in a tumor 3D tumoroid platform using fresh patient tumor tissue.

Methods Tumoroids from fresh RCC tumor samples measuring 150 μm in size were generated using a proprietary mechanical process without any enzymatic digestion or propagation. This study was approved by Vanderbilt University Ethics Board, approval number 031078. An array of multiplexed immunofluorescent panels including lymphoid and myeloid markers were applied at timed endpoints with drug treatments ex vivo to highlight dynamic changes among tumor, immune and stromal components of the TME. Samples were then imaged with confocal microscopy.

Results In this study we analyzed the interaction between the tumor cells (CA9), cancer-associated fibroblasts (FAP), and endothelial cells (CD31) in patient renal cell carcinoma tumoroids. Systematical mapping of the immune landscape in tumoroids treated ex vivo with a STING agonist alone or in combination with nivolumab with a cGAS-STING pathway activation allowed characterization of the spatial and temporal distribution of tumor resident T-cells (CD3, CD4 and CD8), B-cells (CD20), and macrophages (CD68, CD163, and CD11b) in the intact tumor microenvironment. These findings were correlated with treatment-mediated changes in tumor cell killing. To further document treatment-mediated changes in the tumor immune microenvironment, we performed 17-plex cytokine release assays (GM-CSF, sCD137, INFγ, IL4, IL5, IL6, IL10, IL13, MIP1α, MIP1β, TNFa, Perforin) with supernatants collected from ex vivo treated tumoroid cultures.

Conclusions In this comprehensive multiplexed immunofluorescence 3D tumoroid study we demonstrated that the 3D-EXplore ex vivo platform supports an intact tumor microenvironment, ideal for monitoring treatment-induced changes in the tumor resident immune cell populations and their interaction with the epithelial and stromal components of the tumor.

Ethics Approval All tissues in this study were collected under proper patient consent and approved by the Institutional Ethics Board, approval number Pro00014313