INTERROGATION OF THE TUMOR IMMUNE MICROENVIRONMENT AND EX VIVO PROFILING OF PD-1 BLOCKADE USING THE 3D-EXPLORE EX VIVO PLATFORM OF FRESH PATIENT TUMOR TISSUE
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Background The patient derived 3D-EXplore ex vivo platform that incorporates features of the tumor microenvironment provides the multifaceted approach required to model the dynamic response to immune checkpoint blockade. For many patients resistance to PD-1/PD-L1 blockade remains a challenge and new approaches are needed to guide treatment. Here, we demonstrate feasibility of the 3D-EXplore platform utilizing fresh patient tumoroids to interrogate the impact of PD-1 blockade on the tumor immune microenvironment in Non-Small Cell Lung Cancer (NSCLC).

Methods Tumoroids measuring 150 μm in size were generated using a proprietary mechanical process without any enzymatic digestion or propagation from fresh NSCLC samples (n=70). This study was approved by Vanderbilt University Ethics Board; approval number 031078 and Ohio State University Ethics Board: 2014J0130. 3D-EXplore ex vivo studies were performed with anti-PD-1 monoclonal antibodies nivolumab or pembrolizumab and multiparameter flow cytometry analysis was carried out to assess the treatment-mediated changes in the tumor resident T lymphocytes, Tregs, NK/NKT and myeloid cell populations. Furthermore, we performed multiplex cytokine release assays with supernatants collected from the ex vivo treated tumoroid cultures.

Results Multiplex flow cytometric analysis demonstrated the heterogeneity of the tumor immune cell populations including myelomonocytic and leukocyte lineages in different patient tumor samples. 3D ex vivo samples treated with nivolumab and pembrolizumab demonstrated PD1 occupation on CD3 T-cells, while approximately 20% of tumors showed increased CD8 T-cell activation upon ex vivo treatment that correlated with proinflammatory cytokine release in the conditioned media. The ex vivo response to PD1 inhibitors was correlated with changes in the expression of other immune checkpoint proteins, such as CTLA4, immune scoring, tumor mutation burden, tumor pathologic stage, and PD-L1 expression assessed by E1L3N IHC in FFPE tumor sections.

Conclusions Our data demonstrated that 3D-EXplore is as a clinically relevant platform for examining the efficacy of immunotherapeutic interventions. As shown here, PD-1 blockade had tumor and donor specific treatment efficacies with respect to overcoming the suppressive tumor environments. Furthermore, the 3D-EXplore platform provides keen insight into the intact tumor microenvironments, facilitating the development and efficacy of immunotherapeutic agents in cancer.

Ethics Approval All tissues in the study were collected under patient consent approval by Vanderbilt University Ethics Board; approval number 031078 and Ohio State University Ethics Board: 2014J0130