IN VITRO POTENCY ASSAYS FOR IMMUNE CHECKPOINT BLOCKADE USING HUMAN PRIMARY CELLS, MURINE HUGEMM IMMUNE CELLS AND PATIENT-DERIVED TUMOR ORGANOIDS

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Background The demand of evaluating potency of immune checkpoint modulators is steadily growing for immune-oncology drug development.

Methods We aimed to establish a platform to assess the effects of immune checkpoint blockade using human primary immune cells, humanized murine primary immune cells, and co-cultures of tumor cells or patient-derived tumor organoids with immune cells.

Results First, we validated the potency of immune checkpoint blockade, such as anti-PD-1 antibodies, using mixed lymphocyte reaction (MLR) assay and T cell activation assay by in vitro stimulation. Secondly, we introduced tumor cell lines into co-culture system with immune cells and validate the potency assay by measuring cytokine production and tumor cell killing by allogenic T cells. Thirdly we used huGEMM mouse-derived immune cells to replace human primary immune cells in potency assays. HuGEMM mice express engineered human immune checkpoint targets on immune cells and they can serve as an excellent resource of primary immune cells to test the drug candidates targeting human checkpoints in vitro. Last, we developed a patient-derived tumor organoid co-culture system with immune cells. We profiled the expression of immune inhibitory molecules on the tumor organoids and assessed the potency of immune checkpoint inhibitors.

Conclusions In summary, we have established an extensive in vitro platform to evaluate the potency of the next generation of immune checkpoint inhibitors.

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MOLECULAR DISSECTION OF TUMOR-IMMUNE MICROENVIRONMENT FACTORS ASSOCIATED WITH RESPONSE TO CHECKPOINT INHIBITOR THERAPY IN NON-SMALL CELL LUNG CANCER PATIENTS USING NANOSTRING DIGITAL SPATIAL PROFILING

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Background Understanding the dynamics of immune cells in the lung tumor microenvironment following immune checkpoint inhibitor (ICI) therapy is important for developing therapies tailored to patients with progressive disease. We sought to characterize protein and mRNA biomarkers in the tumor and stromal microenvironment in such patients with the Nanostring Digital Spatial Profiling (DSP) platform. DSP technology allows highly multiplexed profiling of proteins and RNA in a spatially resolved manner.

Methods FFPE non-small lung adenocarcinoma biopsies from 18 patients were sourced commercially (Capital Biosciences, MD). Patients had surgical resection of tumors then adjuvant chemotherapy. Upon progression, patients received monotherapy ICI (nivolumab or pembrolizumab). Once progressed on ICI, biopsies were performed and patients were then treated with platinum-doublet or single agent chemotherapy and followed until progression and/or death. Best overall response (BOR) and progression free survival (PFS) was available for ICI. FFPE tumors were sectioned and stained with anti-Pan-Cytokeratin, anti-PDL1 and anti-4-1BB (CD137) using standard immunofluorescence techniques. Twelve circular regions of ~400 um in diameter containing tumor (PanCK+) and stromal (PanCK-) areas were selected per patient (figure 1). The technology uses a photocleavable DNA barcode strategy to multiplex antibodies and RNA in-situ hybridization probes. The GeoMX instrument was used to generate counts for 58 proteins and 84 RNAs on serial sections. Data normalization, linear modeling and survival analysis was conducted in R.

Results Lymphoid and myeloid markers were more abundant in stroma, indicating the microenvironment is diverse and confirming the DSP platform can segment adjacent cells. High levels of PDL1 protein in the tumor were correlated with T cell markers in the stroma (CD3, CD8, ICOS, IDO, OX40L) and inversely correlated with granulocytic (CD66b) and angiogenesis markers (CD34). We focused outcomes analysis on ICI response (9 PD/9 PR). OX40L protein was higher in patients with progression-free survival of <6 months (p = 0.0065).

Conclusions The Nanostring DSP platform provides unprecedented resolution of tumor-stroma immunity and could be a valuable adjunct to clinical trial analysis.

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