

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

Xuefei Yan, Hongjuan Zhang\*, Jun Zhou, Jia Zheng, Shuang Zhu, Rui Zhang, Mingfa Zang, Annie Xiaoyu An, Xiaoxi Xu, Shuzong Wang, Kevin Qijin Xu, Davy Xuesong Ouang, Henry Li, Yujun Huang. *Crown Bioscience, San Diego, CA, USA*

**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.

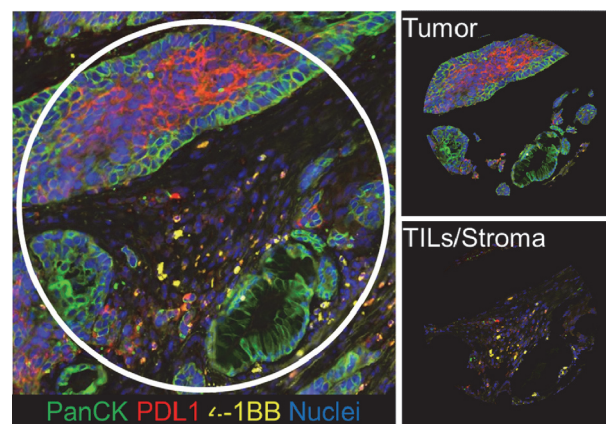
<http://dx.doi.org/10.1136/jitc-2020-SITC2020.0237>

238 **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**

Omar Jabado\*, Suzana Couto, Jordan Blum, Patrick Franken, Patricia Coutinho de Souza, Maria Jure-Kunkel, Nora Pencheva, Brandon Higgs, Kate Sasser, Mark Fereshteh. *Genmab, Princeton, NJ, USA*

**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.

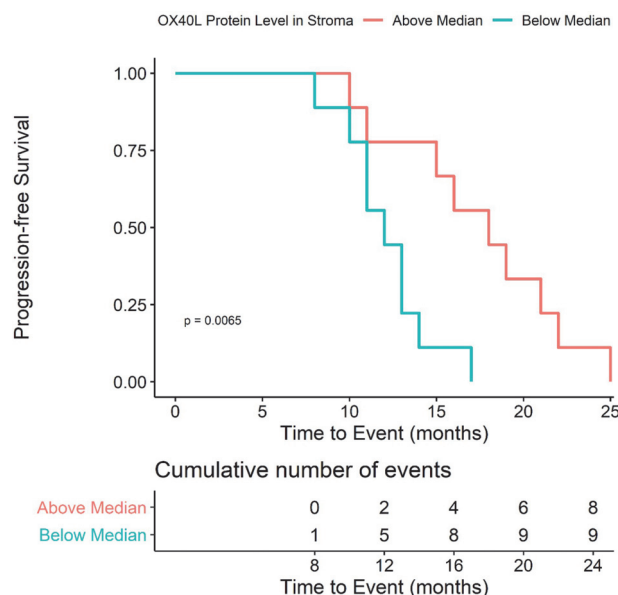
**Methods** FFPE non-small lung adenocarcinoma biopsies from 18 patients were sourced commercially (Capital Biosciences, MD). Patients had surgical resection of tumors then adjuvant chemotherapy. Upon progression, patients received monotherapy ICI (nivolumab or pembrolizumab). Once progressed on ICI, biopsies were performed and patients were then treated



**Abstract 238 Figure 1** Immunofluorescence staining and segmentation of NSCLC tumor

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  $\mu$ m 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



**Abstract 238 Figure 2** Association of OX40L abundance in stroma with PFS using Kaplan-Meier analysis

with partial response and associated with delayed progression (figure 2). CD74 protein was associated with progressive disease during ICI therapy. CSF1R, CD4 and PECAM1 mRNA expression levels in stroma trended with progressive disease.

**Conclusions** In this study we recapitulated the role of OX40L as a marker for response to ICI<sup>1</sup> and CSF1R and PECAM1 in non-response to ICI.<sup>2-3</sup> CD74 is a receptor for the pro-inflammatory cytokine (MIF) however CD74 ectodomain shedding may function as a decoy receptor.<sup>4</sup> These findings highlight how DSP can be used to probe the tumor microenvironment to identify pathways specific to NSCLC non-response for therapeutic target and biomarker development.

**Ethics Approval** Subjects provided informed consent to Capital Biosciences for genetic and protein analysis.

**Acknowledgements** Liang Zhang, Adrienne Whitman, Jennifer Hart, JingJing Gong of Nanostring Technologies.

## REFERENCES

1. Massarelli E, Lam VK, Parra ER, *et al.* High OX-40 expression in the tumor immune infiltrate is a favorable prognostic factor of overall survival in non-small cell lung cancer. *J Immunother Cancer* 2019;7(1):351.
2. Cannarile MA, Weisser M, Jacob W, Jegg AM, Ries CH, Rüttinger D. Colony-stimulating factor 1 receptor (CSF1R) inhibitors in cancer therapy. *J Immunother Cancer* 2017;5(1):53.
3. Kuang BH, Wen XZ, Ding Y, *et al.* The prognostic value of platelet endothelial cell adhesion molecule-1 in non-small-cell lung cancer patients. *Med Oncol* 2013;30(2):536.
4. Schröder B. The multifaceted roles of the invariant chain CD74-More than just a chaperone. *Biochim Biophys Acta* 2016;1863(6 Pt A):1269–1281.

<http://dx.doi.org/10.1136/jitc-2020-SITC2020.0238>

239

## DEC2 LOSS PROMOTES RESISTANCE OF TUMOR CELLS TO IMMUNOTHERAPY BY AFFECTING CD8+ T CELL-REGULATED TUMOR FERROPTOSIS

Thomas Gajewski, Emily Higgs, Shuyin Li\*, Blake Flood, Ken Hatogai. *The University of Chicago, Chicago, Illinois, USA*

**Background** Checkpoint blockade therapies have transformed the landscape of cancer care. Durable clinical responses have been observed in a subset of patients. However, many patients do not respond, and understanding the mechanisms that determine tumor resistant to checkpoint blockade drugs could potentially benefit more patients. Ferroptosis is a relatively newly described form of regulated cell death distinct from apoptosis and necroptosis. Recently, T cell-promoted tumor ferroptosis was shown to be an anti-tumor mechanism and targeting this pathway could be a potential therapeutic approach.

**Methods** To identify genes critical to immunotherapy resistance, B16.SIY cells were transduced with a genome-scale gRNA lentivirus to generate loss of function mutants. In vitro-primed CD8+ T cells isolated from 2C/Rag2-/- TCR transgenic mice specific for the SIY antigen were co-cultured with transduced B16.SIY tumor cells. Resistant mutants were identified by sequencing the gRNAs of survival clones. The gene encoding Decr2, a peroxisomal 2,4-dienoyl-CoA reductase, was identified. To investigate the role of Decr2 in tumor growth and immune responses in vivo, the Decr2 knock-down or Decr2 overexpressed tumors were transplanted into B6 mice and the mice were subsequently treated with anti-PD-L1 antibody. The tumor microenvironments were analyzed by flow cytometry. To understand the resistance mechanism of Decr2 knock-down tumors, RNA-seq was performed and

analyzed. The CD8+ T cell mediated tumor ferroptosis in vitro and in vivo was analyzed for lipid reactive oxygen species.

**Results** Decr2 mutants were relatively resistant to CD8+ T cell killing in vitro. Consistent with this resistance to CD8+ T cell killing, Decr2 knock-down tumors showed minimal response to anti-PDL1 therapy in vivo. RNA-seq analysis of Decr2 knock-down B16.SIY tumors revealed upregulation of ferroptosis-related genes, including slc7a11. Further mechanistic studies showed that Decr2 knock-down tumors displayed defects in ferroptosis in vitro and in vivo.

**Conclusions** Decr2-deficient tumors were relatively resistant to CD8+ T cell killing in vitro and anti-PD-L1 immunotherapy in vivo by modulating CD8+ T cell-induced ferroptosis.

<http://dx.doi.org/10.1136/jitc-2020-SITC2020.0239>

240

## IDENTIFICATION OF LUNG CANCER MUTATIONAL SIGNATURES AND TUMOR DRIVERS ASSOCIATED WITH SPECIFIC BIMODAL PD-L1/TMB STATUS

Fernando Lopez-Diaz\*, Lauryn Keeler, Sally Agersborg, Lawrence Weiss, Vincent Funari. *Neogenomics Laboratories, Carlsbad, CA 92008, CA, USA*

**Background** PD-L1 expression and Tumor Mutation Burden (TMB) have independently emerged as prospective biomarkers of response to anti PD1-/PDL1 checkpoint inhibitors and even combined use of TMB, PD-L1 protein levels has been proposed. However, how the tumor genomic landscape interplays with the tumor microenvironment (TME) in defining particular predictive therapy response statuses is not clear.

**Methods** 424 FFPE clinical samples from lung cancer patients were analyzed using a CLIA-validated NGS-based assay that interrogates SNVs, indels using a 323 gene panel and by IHC for PD-L1 using the FDA approved PharmDx assay. TMB (mutations/Mb) is categorized as low ( $\leq 7$ ), intermediate (7-15). NGS results were paired with PD-L1 status which was defined by tumor proportion scores (TPS) as: negative (TPS<1%), Low expressing ( $\geq 1-49\%$ ) and High ( $\geq 50\%$ ). In silico analyses were also performed on 5939 lung cancer samples from public databases.

**Results** We found poor correlation between PD-L1 expression and TMB in NSCLC ( $r^2=0.266$ ). We then classified lung cancer samples based on TMB and PD-L1 TPS and found mutational correlations specific to in each of the groups defined by PD-L1 combined with TMB scores. First, we interrogated the KRAS and EGFR mutations frequencies distribution across either TMB or PDL1 status. We find that while KRAS mutations are constant across PDL1 TPS but infrequent on TMB High tumors, EGFR mutation frequency appeared inversely correlated to both TMB and PD-L1 TPS. 67% of PD-L1 High/TMB Low samples presented mutations either on EGFR (12%), KRAS (23.5%) or in genes from known driver TRK/MAPK pathways, whereas only KRAS was part of the frequently mutated gene signature with 36.5% (13/36) samples mutated on PD-L1 High/TMB High samples. Neither EGFR nor KRAS were found frequently mutated on PD-L1 Low/TMB High group (n=46). Interestingly in patients with an intermediate TMB (7).

**Conclusions** Genomic alteration signatures might define subsets of lung cancer tumors with no PD-L1 expression to complement TMB and PD-L1 on the selection criteria for patients whom may benefit from checkpoint inhibitors.

**Acknowledgements** Paris Pettersen, Hatim Husain.