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\(^1\) and CSF1R and PECAM1 in non-response to ICI.\(^2\) CD74 is a receptor for the pro-inflammatory cytokine (MIF) however CD74 ectodomain shedding may function as a decoy receptor.\(^4\) 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.

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239 DECR2 LOSS PROMOTES RESISTANCE OF TUMOR CELLS TO IMMUNOTHERAPY BY AFFECTING CD8+ T CELL-REGULATED TUMOR FERROPTOSIS

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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.

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

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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 (≤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 (1–49%) and High (≥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=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 PD-L1 status. We find that while KRAS mutations are constant across PD-L1 TPS but infrequent on TMB High tumors, EGFR mutation frequency appeared inversely correlated to both PD-L1 and 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.

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