Background Targeting immunosuppressive checkpoints has proven to be an efficacious treatment strategy for non-small cell lung cancer (NSCLC), in which response rates are as high as 35% in patients harboring Kras/p53 (KP) mutations.1 However, most patients demonstrate no or only partial response to immune checkpoint blockade (ICB), underscored by the need to better understand the suppressive mechanisms in the tumor-immune microenvironment. Murine models of KP mutant lung cancer demonstrate upfront sensitivity to PD-1 checkpoint blockade but rapidly acquire resistance2, providing useful tools to discover tumor-intrinsic mechanisms of resistance.

Methods We generated new KP syngeneic and autochthonous lung tumor models with intrinsic resistance to anti-PD-1 treatment via in vivo passaging in the face of ICB treatment. Additionally, we analyzed transcriptome data from anti-PD-L1 treated KP tumors, identified differentially expressed genes between response and resistance timepoints, and queried these in the newly generated anti-PD-1 resistant tumor models. We utilized flow cytometry to characterize the tumor-infiltrating immune microenvironment with manipulation of candidate gene expression.

Results Our data identified a stable upregulation of the phosphodiesterase enzyme, autotaxin (ATX), and the metabolite that it generates, lysophosphatidic acid (LPA), in ICB resistant tumors. Analyses of lung adenocarcinoma patient datasets revealed a significant positive correlation between ATX and immune signatures, including a previously published dataset encompassing immune checkpoints and suppressive molecules3, suggesting that ATX is upregulated in the face of normal anti-tumor immunity. Mechanistic studies utilizing isogenic pairs of tumors demonstrated that ATX expression inversely correlated with CD8+ T cell proliferation and cytotoxic functionality, with ATX-overexpression in anti-PD-1 sensitive tumors promoting intrinsic resistance. We next sought to define LPA receptor (LPA5) expression on T cells. Flow cytometry on tumor-infiltrating CD8+ T cells demonstrated significantly altered expression of LPAR5 within anti-PD-1 resistant versus sensitive tumors, with upregulation of LPAR5 and downregulation of LPAR2. Additionally, analysis of previously published RNA-sequencing of CD8+ T cells from NSCLC patients4 revealed that lower LPAR2 versus LPAR5 correlated with worse response to ICB. Importantly, targeting ATX or LPAR5 with PD-1 blockade caused significant tumor regressions in clinically relevant models of KP mutant lung cancer via interrogation of anti-tumor CD8+ T cells.

Conclusions Our data indicate that increased ATX/LPA activity occurs downstream of immune activation, which in turn stimulates LPAR5 signaling on CD8+ T cells to diminish cytolytic functions. These results provide evidence that this axis acts as an immunosuppressive checkpoint, providing rationale that co-targeting it with ICB should improve anti-tumor immune response.

References