## 948 AXL EXPRESSION ON DCS SUPPRESSES ANTI-TUMOR IMMUNITY

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**Background** Tumor cells exploit immune checkpoints to evade immune detection and hinder immune cell activation. Despite the clinical implications of immune checkpoint blockade, resistance remains a challenge. Axl, a receptor tyrosine kinase, has been shown to suppress production and signaling of pro-inflammatory cytokines in response to inflammatory signals on innate immune cells. In this study, we describe that Axl may serve as an immune checkpoint on dendritic cells (DCs) during antitumor immune responses.

**Methods** Tumor cells with different levels of immunogenicity MC38 colon, B16F0-cOva melanoma and Py8119-cOva breast cancer cells were evaluated for tumor growth delay and tissue evaluation after subcutaneous implantation into C57BL/6J mice. Tumors from WT (Wild Type) and Axl knockout (KO) mice were harvested and processed into single cell suspensions 7 days post inoculation. Additional, genotypes were crossed to evaluate the role of host Axl that included Batf3 knockout, IFNR knockout, Axlflox/flox, CD11cCre, and LysMCre. Tumor infiltrating leukocytes (TIL) were enriched via magnetic bead isolation and single cell RNA sequencing was performed using the 10x Genomics Chromium platform. T cell depletion and IFNAR1 blockade was respectively achieved via the intraperitoneal or intratumoral antibody administration. Established tumors were treated with a single intratumoral dose of DMXAA with or without a 15 Gy dose of ionizing radiation.

**Results** We observed that Axl ablation in KO mice led to tumor growth delay in syngeneic tumor models MC38, B16F0-cOva and Py8119-cOva. We hypothesized this was immune related, and to investigate TIL functional states and cellular interactions we performed single cell RNA sequencing of TILs from dissociated MCD38 and B16F10-cOva. We observed increased antigen activation programs in T cells, increased T cell priming capability, and type-I interferon (IFN) signaling in DCs of AXL KO mice compared to WT. Delayed tumor growth in AXL KO host was then shown to be dependent on Batf3+ DCs and cytotoxic CD8 T cells. Ablation of AXL on DCs but not on macrophages or neutrophils, was sufficient to enhance the anti-tumor response, that was dependent on type-I IFN signaling. The combination of Axl ablation with DMXAA, a STING agonist, known to promote type-I IFN production, resulted in improved therapeutic efficacy. Furthermore, the addition of radiotherapy enhanced responses in the therapy-resistant Py8119-cOva tumors.

**Conclusions** Our findings highlight an important role of Axl on DCs in suppressing CD8 T cell mediated anti-tumor immune responses by limiting type I IFN signaling. These data suggest that AXL is a promising target for combination therapies.

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**Ethics Approval** All mice were housed in specific pathogen free faculties at UTSW Animal Resource Center and treated in accordance with the animal experimental guidelines approved by the Institutional Animal Care and Use Committee (Protocol# 2017–102240).

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