981 AXL-TARGETED MACROPHAGE PHENOTYPE SWITCHING MEDIATES CHECKPOINT-RESISTANCE IN MELANOMA

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Background Anti-PD-1 refractory melanoma remains a devastating clinical challenge. We previously defined AXL tyrosine kinase as an emerging target of interest in tumor associated macrophage (TAM) behavior and immunotherapeutic resistance. Here, we aim to define the mechanisms by which AXL mediates tumor-immune crosstalk and capacity for AXL inhibition to address resistance to checkpoint blockade.

Methods Characterization of mouse melanoma: YUMM1.7 (anti-PD-1 resistant), YUMMeR1.7, B16F10, house-derived metastatic LN6, and RAW macrophages was performed with AXL ligand stimulation or inhibitors warfarin and bemcetinib. Co-cultures examined tumor cell versus macrophage-derived AXL impact on migration, efferocytosis, antigen presentation, and cytotoxic T-cell function. In vivo models used AXL inhibition as single agent or in combination with Anti-PD-1. CSF1R and F4/80 myeloid depletion strategies explored macrophage-dependent AXL function. Flow cytometry, immune FRET (iFRET), and serum luminex were used for comprehensive immune profiling. Blood from melanoma patients was evaluated for sAXL and monocyte differentiation assays. TCGA-SKCM melanoma tumor mRNA expression and clinical data were downloaded from the GDC legacy archive and public single cell RNA melanoma datasets.

Results Serum sAXL increased by stage and was associated with PD-1 nonresponse in Stage IV patients ($p<0.01$). AXL expression diverged from T-cell signatures but was associated with immunosuppressive myeloid signatures ($p<0.001$). Single-cell data revealed complex overlap in M1/M2 macrophage plasticity that diverged amongst metastatic and primary tumors, however, AXL expression was significantly elevated in unique Angio-TAMs(VEGF+, SPP1+) and regulatory-TAM cell-chat pathways. In vitro, tumor migration was increased with Gas6 and inhibited by warfarin for AXL positive lines ($P<0.05$). GAS6 stimulation increased macrophage proliferation and immunosuppressive (M2) phenotype that was reversed with AXL inhibition($p<0.05$). M2 media stimulated tumor migration whereas AXL inhibition promoted apoptosis. In vivo, AXL inhibition decreased YUMM1.7 growth and sensitized tumors to anti-PD-1($p<0.02$). YUMMer1.7 tumors spontaneously regressed and immunized mice against re-challenge with YUMM1.7. Macrophage-depleted tumors were refractory to AXL inhibition($p<0.05$), however CSF1R-inhibited tumors were larger than controls ($p<0.05$). iFRET confirmed increase in PD-1 receptor engagement with warfarin treatment. Flow cytometry confirmed M1 macrophage expansion with AXL inhibition strategies with correlating shifts in M2 secreted factors(IL-10, M-CSF, VEGF).

Conclusions Our data indicate myeloid-specific effects of AXL activity in tumors are targetable in PD-1 refractory melanoma. Analysis is ongoing to define spatial intratumoral, intranodal, and geographic intertumoral heterogeneity. Our data provide mechanistic insight to the potential for macrophage-specific and AXL-directed therapy to improve immunotherapeutic response and further exploration of potential as a predictive biomarker is warranted.

Ethics Approval All human and mouse studies were conducted with IRB and APLAC approval (IRB 68118, APLAC 34156). Informed patient consent was obtained for tissue and biobanking.

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