1224 CELL-SPECIFIC NANOENGINEERING STRATEGY TO DISRUPT TOLEROGENIC SIGNALING FROM MYELOID-DERIVED SUPPRESSOR CELLS AND INVIGORATE ANTITUMOR IMMUNITY IN PANCREATIC CANCER

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Background A defining hallmark of immunosuppression in pancreatic ductal adenocarcinoma (PDAC) is the frequent trafficking of neutrophilic/granulocytic myeloid-derived suppressor cells (gMDSC) which exert their tolerogenic anti-T-cell functions through multiple mechanisms, particularly STAT3-mediated arginase-1 (Arg1) activity. Systemic inhibition of gMDSC trafficking and/or function (e.g., CXCR2, TGF-β, etc.) has been disappointing due to neutropenia, compensatory myelopoietic adaptations, and off-target effects. We sought to design a novel nanoengineering strategy to abrogate tolerogenic signaling in a gMDSC compartment-specific manner.

Methods We chemically modified AZD5069—a small-molecule inhibitor of the gMDSC surface receptor CXCR2—by conjugating it with polyethylene glycol (PEG) to enhance aqueous solubility. This AZD5069-PEG construct was chemically grafted on an amphiphilic polysaccharide derivative to engineer AZD5069-decorated nanoparticles (NP^{CXCR2}). We encapsulated hydrophobic STAT3i Ruxolitinib in NP^{CXCR2} nanoparticles and compared its effect on inhibition of Arg1 activity from gMDSCs and T-cell activation *in-vitro* and *in-vivo*.

Results To confirm CXCR2 as a gMDSC-specific target, we identified exclusive expression of CXCR2 in gMDSCs in human and murine PDAC via single-cell RNA sequencing and flow cytometric analysis in peripheral blood mononuclear cells derived from treatment-naïve PDAC patients (n=57). NP^{CXCR2} loaded with Cy5.5 dye showed dramatically higher uptake in gMDSC-like promyelocytic J774 cells compared with other PDAC-relevant cells *in-vitro*— KPC6694c2 tumor-cells, cancer-associated fibroblasts (CAF), and M0 macrophages RAW274.1. In *in-vivo* orthotopic $K\text{-}ras^{LSL.G12D/+};p53^{R172H/+};Pdx1^{Cre/+}$ (KPC) tumor cell:CAF co-injection models, although Cy5.5 dye-loaded non-AZD5069 decorated NP^{CTL} and NP^{CXCR2} both trafficked to tumor sites equally, NP^{CXCR2} but not NP^{CTL} constructs preferentially concentrated in $F4/80^{-}Ly6G^{+}$ gMDSCs compared with $F4/80^{+}$ macrophages, $F4/80^{-}Ly6C^{+}$ monocytic MDSCs, $EpCAM^{+}$ tumor cells, $PDPN^{+}$ CAFs, $CD3^{+}$ T-cells, and $Cd11c^{+}$ dendritic cells by flow cytometry. Encapsulation of STAT3i Ruxolitinib in NP^{CXCR2} and treatment of endogenous $pSTAT3^{hi}$ J774 cells *in-vitro* showed significantly more durable inhibition of pSTAT3 compared with Ruxolitinib drug treatment alone. Consequently, given that JAK2/STAT3 signaling is the major regulator of Arg1 activity in gMDSCs, Ruxolitinib-NP^{CXCR2} treatment of J774 cells significantly reduced gene expression and enzymatic activity of Arg1 compared with free Ruxolitinib treatment. Co-culture of splenocyte-derived murine $CD8^{+}$ T-cells with J774 treated with Ruxolitinib-NP^{CXCR2} showed significant improvement in T-cell IFN-γ release compared with NP^{CTL} or free Ruxolitinib-treated co-culture conditions. In orthotopic KPC tumor-bearing mice, intravenous delivery of NP^{CXCR2}-encapsulated Ruxolitinib significantly augmented intratumoral trafficking of $IFN\text{-}\gamma^{+}CD107a^{+}$ $CD8^{+}$ T-cells, compared with free Ruxolitinib treatment, without appreciable systemic neutropenia *in-vivo*.

Conclusions Cell-specific delivery of payloads via CXCR2-homing nanoparticles represent a novel immunotherapeutic strategy to target tolerogenic signaling pathways in gMDSCs and invigorate antitumor immunity in PDAC.

Ethics Approval All animal experiments were performed in accordance with the NIH animal use guideline and protocol 21-176 approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Miami.

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