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SYSTEMIC ADMINISTRATION OF AXA-042, A NOVEL TLR2/6 AGONIST, RESHAPES THE TUMOR MICROENVIRONMENT, AS REVEALED BY SINGLE CELL SEQUENCING ANALYSIS

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Background Treatment approaches that engage both the innate and adaptive immune response have the potential to transform anti-cancer therapy, especially in settings of checkpoint inhibitor insensitivity. Toll-like receptors (TLRs) mediate the initial cellular response to external pathogens or endogenous alarmins, activating downstream pro-inflammatory cascades associated with innate cell activation and recruitment. AXA-042 is a novel synthetic TLR2/6 agonist designed for systemic delivery to re-engage the innate immune response to help overcome tumor immune escape. Once-a-week treatment with AXA-042 led to 87% growth inhibition of syngeneic EMT6 tumors. Single cell sequencing analysis was completed to characterize the immediate impact of systemic TLR2/6 activation on the tumor microenvironment.

Methods Female Balb/c mice bearing orthotopic EMT6.5 mammary tumors received a single intravenous treatment of saline or AXA-042 (10 µg/mouse). Equal numbers of viable cells were recovered by flow cytometry from collagenase-digested tumors (n=3/group) 24 hours after treatment and pooled prior to loading onto a Chromium Single Cell Chip. 10x Genomics single-cell transcriptome libraries were prepared from each sample and sequenced with Illumina NextSeq 550 sequencing platform. Cell type annotation based on the ImmGen database was determined using the SingleR package. Differential gene expression analyses were completed to identify AXA-042 responsive gene signatures in cell subsets that included at least 10 cells. The identified genes were required to be expressed in at least 3 cells.

Results AXA-042 treatment decreased the total number of tumor-associated macrophages, monocytes, fibroblasts, endothelial, epithelial and stromal cells within 24 hours of treatment. A parallel increase in neutrophils, B cells, NKT and activated CD4 and CD8 T cells was observed. Re-clustering of myeloid populations revealed AXA-042-induced shifts in the macrophage, monocyte and neutrophil clusters. Differential expression analysis within the TLR2 positive myeloid cells demonstrated AXA-042-mediated engagement of pathways associated with antigen processing and presentation, Fc receptor signaling and immune hypersensitivity in monocytes; granulocyte chemotaxis, lipid metabolism and iron sequestration in neutrophils; and cellular response to bacterial lipopeptide, stress response to metal ions and arginine metabolism in macrophages

Conclusions Systemic AXA-042 treatment led to a reorganization of the tumor microenvironment within 24 hours of treatment. AXA-042 altered the myeloid cell subset profiles, promoted the influx of activated T cells and reduced tumor cell viability. AXA-042 has completed GLP toxicology studies and is currently undergoing evaluation in a Phase 1 clinical trial (ACTRN12622000993796) in advanced solid tumors.

Trial Registration ACTRN12622000993796

Ethics Approval The study was approved by the Austin Health Animal Ethics Committee, approval number 05640.