Targeting Innate Immune Cells for the Treatment of Cancer

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Introduction

- Innate immune cells contribute to tumor immunosuppression by expressing immune checkpoint protein ligands, depleting essential amino acids such as arginine, and producing immune suppressive cytokines such as IL-10.
- Multiple signaling pathways contribute to maintaining the immunosuppressive phenotype of intra-tumoral innate immune cells:
  - Adenosine, found in high concentrations in tumors, suppresses immune cell activation by acting on adenosine receptors, $A_{2a}R/A_{2b}R$, expressed on innate immune cells and lymphocytes.
  - PI3Kγ signaling is central in the decision between an anti-inflammatory pro-tumor M2 macrophage and a pro-inflammatory anti-tumor M1 macrophage.

Methods

- **Monocyte-derived dendritic cells (moDC) and macrophages:** moDC were differentiated from CD14$^+$ monocytes with IL-4/GM-CSF +/- adenosine +/- AB928.
- **Macrophages:** Macrophages were differentiated from CD14$^+$ monocytes with M-CSF. Macrophages were polarized into M1 macrophages with LPS+IFN-γ +/- IPI-549 +/- adenosine +/- AB928. M2 macrophages were polarized with IL-4.
- Macrophages or moDC were then taken for gene expression analysis, NanoString analysis, or placed in a mixed lymphocyte reaction (MLR).
- **T cell Activation Assay:** T cells were activated (αCD2/3/28) in the presence of adenosine with or without AB928 or hArg I with or without A0304567. IFN-γ was measured in the supernatant.
- **Inhibitors used:** AB928 is a dual $A_{2a}R/A_{2b}R$ antagonist. A0304567 is an arginase I inhibitor. IPI-549 is a PI3Kγ inhibitor. All compounds were synthesized by Arcus Biosciences

Expression of Adenosine Receptors, PI3Kγ, and Arginase I in Myeloid Cells

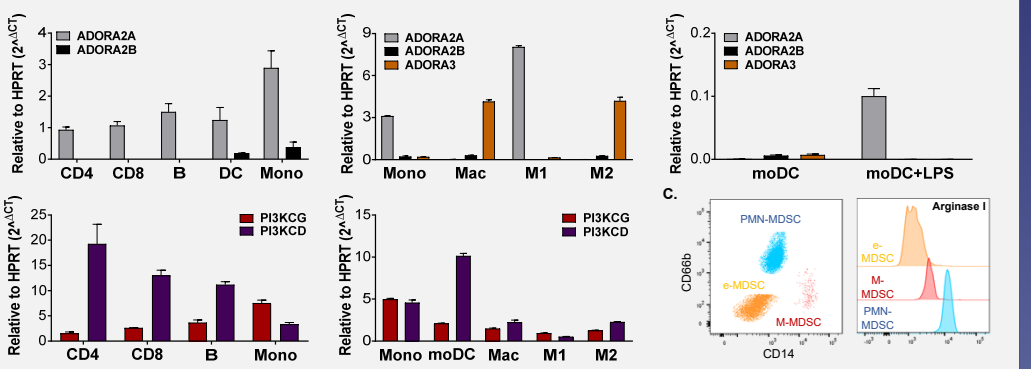

Figure 1. A) Expression of adenosine receptors $A_{2a}R$ (ADORA2A) and $A_{2a}R$ (ADORA2B) at steady state (left) and in differentiated macrophages (middle) and moDC (right). B) PI3KCG and PI3KCD at steady state (left) and in differentiated macrophages and moDC (right). C) Expression of Arginase I in MDSC subsets. Left panel: MDSC subsets gated from HLA-DR$^-$CD33$^+$ PBMC. Right panel: Arginase I expression in MDSC subsets.

Inhibition of A_2R or Arginase I Restores T Cell Activation

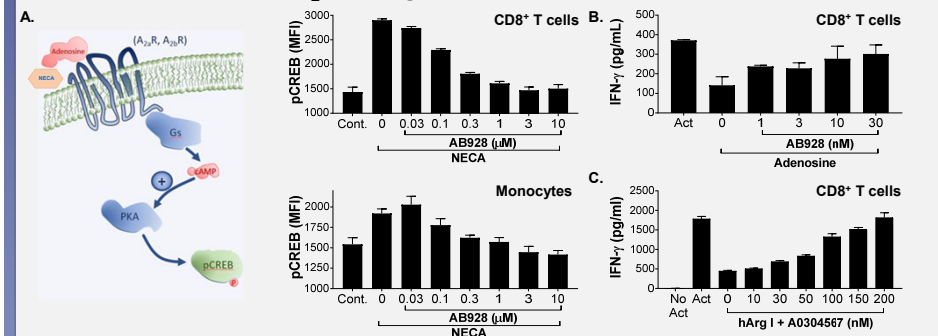

Figure 2. A) A_2R agonist (NECA) stimulates phosphorylation of CREB (pCREB) in CD8$^+$ T cells and monocytes. Addition of AB928 limits A_2R activation as measured by pCREB. B) AB928 rescues the decrease in IFN-γ after adenosine treatment in CD8$^+$ T cells. C. Recombinant arginase I suppresses IFN-γ production and is rescued by A0304567 in CD8$^+$ T cells.

AB928 Inhibits Suppressive Effects of Adenosine During moDC Maturation

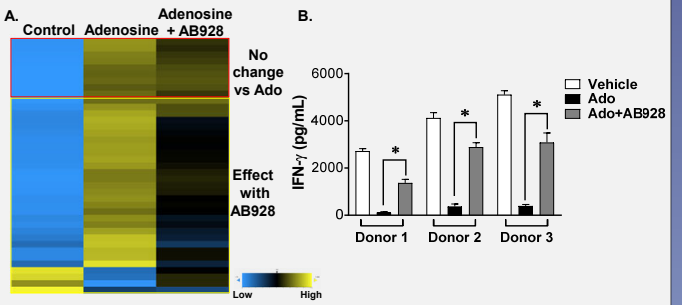

Figure 3. A) Heat map displaying differentially expressed genes in moDC differentiated with adenosine +/- AB928 B) AB928 is capable of restoring IFN-γ levels in an moDC/CD4 MLR. *p<0.05

Adenosine Suppresses the Enhanced M1 Polarization Phenotype Induced by PI3Kγ Inhibition

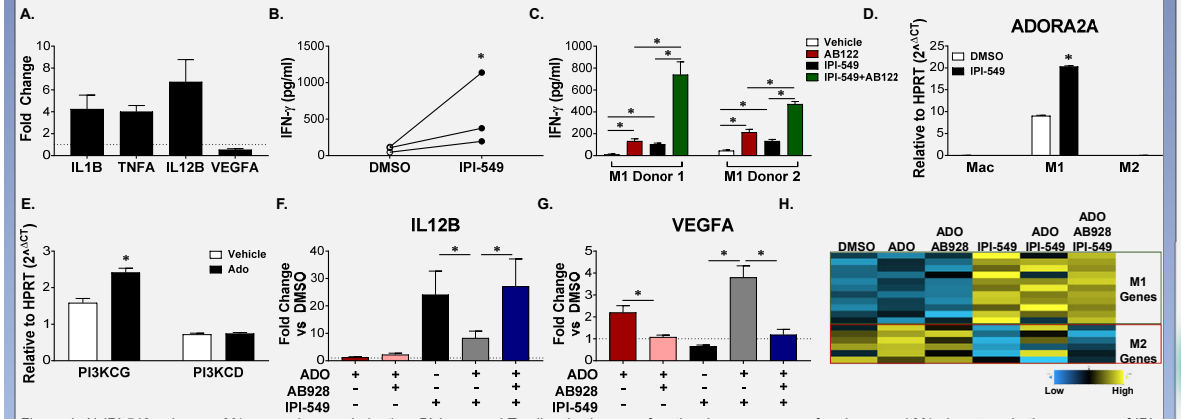

Figure 4. A) IPI-549 enhances M1 macrophage polarization. B) Increased T cell activation as a functional consequence of an increased M1 phenotype in the presence of IPI-549 in an MLR. Three independent donors. C) IPI-549-treated M1 macrophages synergize with AB122 (an anti-PD-1 mAb) for enhanced T cell activation in an MLR. D) IPI-549 increases ADORA2A expression in M1 polarized macrophages. ADORA2B and ADORA3 are unaffected. E) Adenosine increases PI3KCG expression in M1 polarized macrophages. F-G) Adenosine opposes and AB928 rescues the effects of IPI-549 on IL12B (F) and VEGFA (G) in M1 polarized macrophages. H) Heat map displaying differentially expressed genes associated in M1 polarized macrophages +/- IPI-549 +/- adenosine +/- AB928. *p<0.05

Conclusions

- AB928, a dual $A_{2a}R/A_{2b}R$ antagonist, inhibits the immunosuppressive effects of adenosine on immune cells
- PI3Kγ inhibition enhances macrophage M1 polarization and T cell co-stimulatory function
- Cross-talk between PI3Kγ and A_2R receptor signaling observed in M1 polarized macrophages
  - Adenosine upregulates PI3Kγ expression
  - PI3Kγ inhibition upregulates $A_{2a}R$ expression
- Dominant effects of adenosine-mediated inhibition of IL-12 expression even in the presence of IPI-549 is reversible through the addition of AB928.