

# A<sub>2b</sub>R Contributes to Adenosine-Mediated Immunosuppression, Which is Relieved by the Dual A<sub>2a</sub>R/A<sub>2b</sub>R Antagonist AB928

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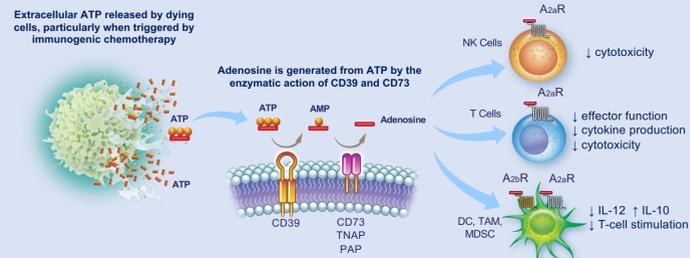
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SITC 2019  
Poster P557

## Introduction

The tumor microenvironment (TME) generates high levels of adenosine (ADO) which binds to A<sub>2a</sub>R and A<sub>2b</sub>R receptors on immune cells to inhibit their activity (Figure 1). AB928 is a selective, small-molecule, dual A<sub>2a</sub>R/A<sub>2b</sub>R antagonist with minimal penetration across the blood brain barrier. It was specifically designed to potentially block the immunosuppressive effects of ADO in the TME. We have previously shown that AB928 blocks the immunosuppressive effects of adenosine in human cultured cells and mouse syngeneic tumors. Four global phase 1/1b disease-specific platform studies are assessing the safety, tolerability, PK, PD, and preliminary clinical activity of AB928 in combination with chemotherapy and/or anti-PD-1 antibody (Powderly et al. ESMO Abstract # 4854 2019).

Herein, we use publicly-available gene expression databases and cell sorting experiments to determine that T cells and other non-myeloid cells predominantly express the A<sub>2a</sub>R adenosine receptor. In contrast, circulating and tumor-resident myeloid cells express both A<sub>2a</sub>R and A<sub>2b</sub>R. We then used monocyte-derived dendritic cells to demonstrate the important role of A<sub>2b</sub>R in moDC activation of CD4<sup>+</sup> T cells and regulation of adenosine-driven gene expression patterns. Next, A<sub>2b</sub>R signaling is shown to regulate gene expression in human non-small cell lung carcinoma (NSCLC) cell lines. Collectively, these studies demonstrate an important role for A<sub>2b</sub>R in adenosine-mediated immunosuppression and provide a mechanistic rationale for stimulation of anti-tumor immune responses with the dual adenosine receptor antagonist AB928, which is currently undergoing evaluation in several Phase 1b clinical trials.



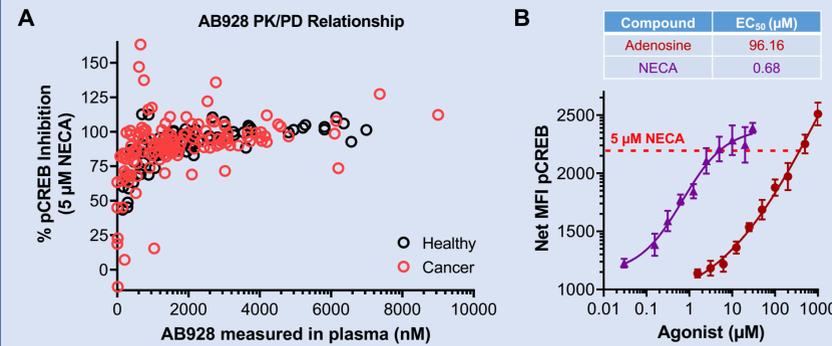
**Figure 1.** Diagram of adenosine production from ATP released into the TME. Hydrolysis of ATP by the ecto-enzymes CD39 and CD73 (PAP, TNAP) produces adenosine, which exerts immunosuppressive effects by binding to adenosine receptors expressed on immune cells.

## Methods

- pCREB:** A fluorochrome labeled monoclonal antibody to phosphorylated CREB was used to measure NECA-driven adenosine signaling in whole blood CD8<sup>+</sup> cells from clinical trial subjects.
- RNAseq data analysis:** Raw counts were downloaded from PanCanAtlas of the The Cancer Genome Atlas (TCGA) (<https://gdc.cancer.gov/about-data/publications/pancanatlas>) and were normalized to log2 counts-per-million (CPM) using edgeR and limma packages in R. Raw fastq files were downloaded from GEO for the Monaco et al dataset, aligned to hg38 human genome using STAR, counts were computed using HTSeq followed by normalization to log2 CPM as described above. The Stand-Up-2-Cancer (SU2C) metastatic castration resistant prostate cancer (mCRPC) dataset was downloaded from cBioPortal (*Abida W, et al. PNAS 2019*) and normalized as above. The hazard ratios were computed using Cox-proportional Hazards model adjusting for significant clinical covariates such as Prostate Specific Antigen (PSA) and Gleason scores.
- moDC experiments:** Monocyte-derived dendritic cells (moDC) were generated from freshly isolated CD14<sup>+</sup> monocytes and differentiated with IL 4/GM-CSF for 6 days +/- adenosine/EHNA +/- antagonists. Cells were then taken for NanoString analysis or placed in a mixed lymphocyte reaction (MLR) with CD4<sup>+</sup> T cells.
- NanoString:** For moDC experiments, RNA was hybridized to the nCounter Immunology Panel (Human V2) codeset and analyzed using nSolver 4.0 software. NSCLC cancer cell lines were hybridized to the nCounter® PanCancer Progression Panel.

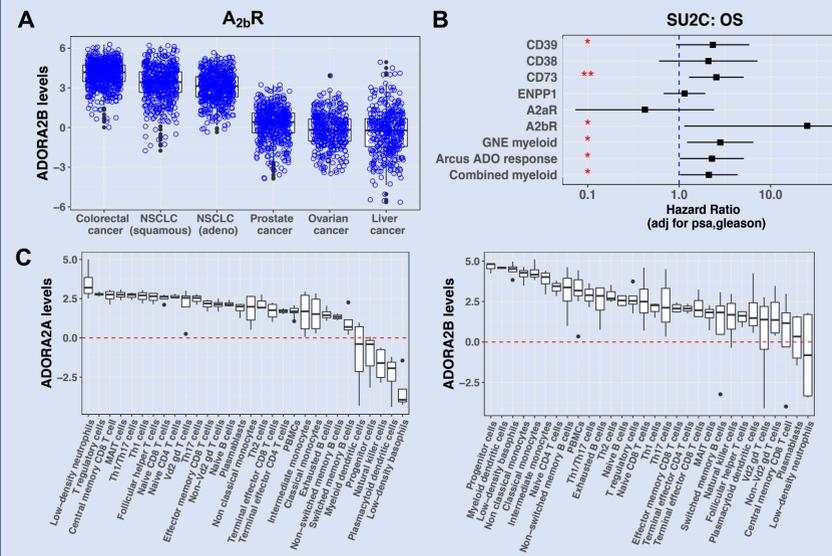
## Results

### AB928 Blocks >100 μM Adenosine Signal Equivalent (5 μM NECA) in Clinical Trial Subjects



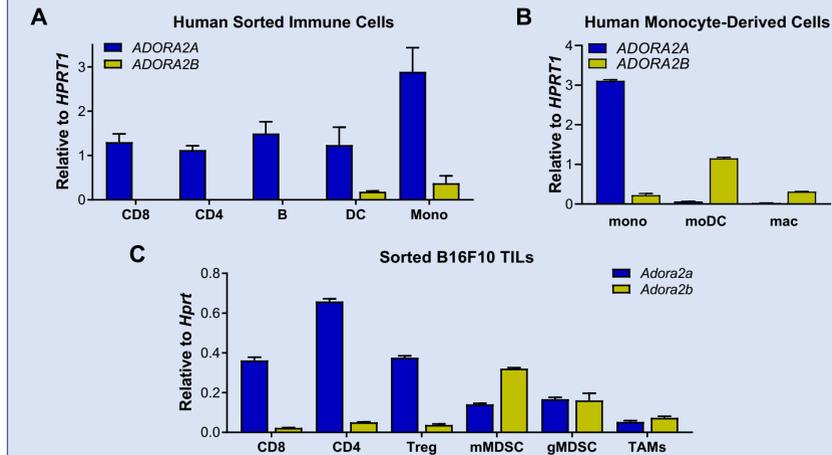
**Figure 2 (A)** PK/PD correlations are shown from oncology subjects enrolled in AB928 combination trials (red circles) and from healthy volunteers (black circles). **(B)** Potency of adenosine and adenosine-receptor agonist, NECA, in stimulating CREB phosphorylation on CD8<sup>+</sup> T cells in human whole blood. Red dashed line denotes the pCREB signal generated by 5 μM NECA.

### A<sub>2b</sub>R is Expressed in Human Tumors and Immune Cells and Correlates with Survival in Metastatic Prostate Cancer



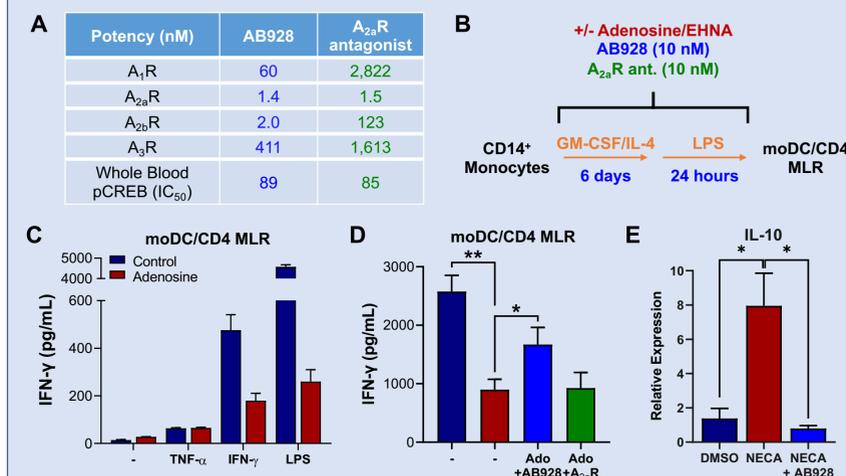
**Figure 3 (A)** A<sub>2b</sub>R (ADORA2B) gene expression (log2 CPM) in colorectal, non-small cell lung and prostate cancers from the PanCanAtlas TCGA dataset. **(B)** Forest plot of the Stand-Up-2-Cancer (SU2C) data set denoting the prognosis of genesets via hazard ratio (*Abida et al 2019*). Stars indicate statistical significance (\*=p<0.1, \*\*=p<0.01). **(C)** A<sub>2a</sub>R (left) and A<sub>2b</sub>R (right) gene expression (log2 CPM) in sorted immune subpopulations from healthy donors (*Monaco et al 2019*).

### Expression of A<sub>2a</sub>R and A<sub>2b</sub>R Adenosine Receptors is Diverse and Cell-Type Specific



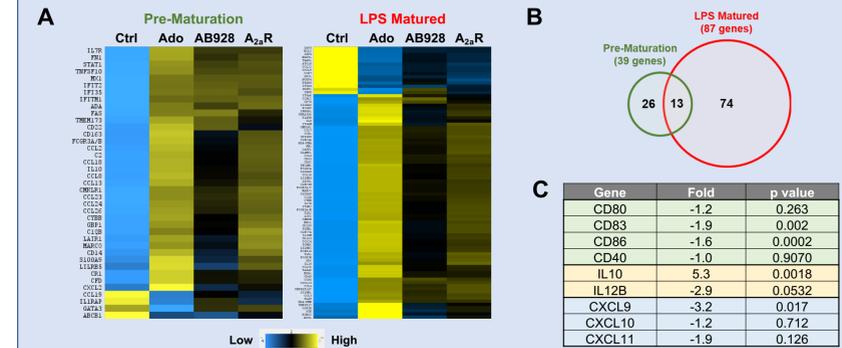
**Figure 4 (A)** Adenosine receptor gene expression of sorted human immune cells from healthy donors. **(B)** Real-time PCR of freshly isolated monocytes as well as monocyte-derived dendritic cells and macrophages following 6 day differentiation *in vitro*. **(C)** Sorted immune cells from mouse B16F10 tumors showing the higher ratio of A<sub>2b</sub>R/A<sub>2a</sub>R gene expression in myeloid cells.

### Dual A<sub>2a</sub>R/A<sub>2b</sub>R Antagonism Recovers Adenosine-Mediated Suppression in moDC



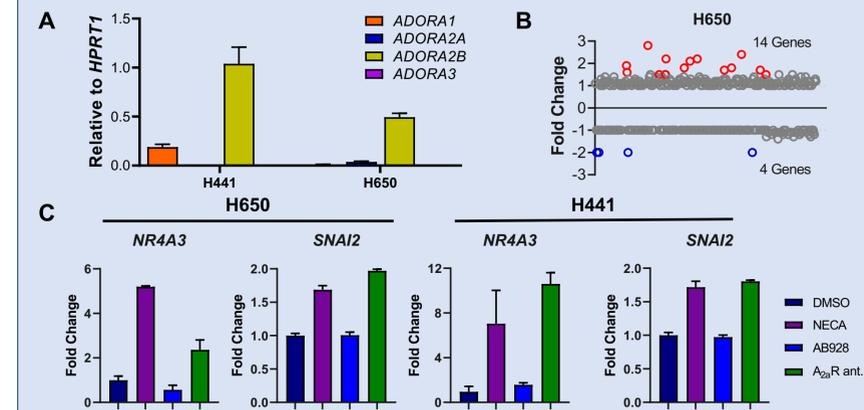
**Figure 5 (A)** Potency of AB928 and a selective A<sub>2a</sub>R antagonist were determined using adenosine receptor over-expressing CHO cell lines and a human whole blood assay. **(B)** moDC experimental design: adenosine and EHNA (adenosine deaminase inhibitor) were incubated +/- antagonists during moDC differentiation. **(C)** Comparison of different moDC maturation stimuli on adenosine inhibition of moDC in a MLR. **(D)** Dual antagonism with AB928 rescues the ability of moDC to stimulate IFN-γ secretion in a MLR. **(E)** Gene expression of IL-10 in primary human CD141<sup>+</sup> DCs incubated with NECA +/- AB928 \*p<0.05, \*\*p<0.01.

### Dual A<sub>2a</sub>R/A<sub>2b</sub>R Antagonism Recovers Gene Expression Changes in moDC



**Figure 6 (A)** Heat map of NanoString data showing differentially expressed (DE) genes (fold change >2.0, p-value <0.05) from moDC. **(B)** Venn diagram of DE genes from moDC before and after LPS maturation. **(C)** Selected gene expression data from LPS matured moDC.

### A<sub>2b</sub>R Signaling in Cancer Cell Lines Drives Gene Expression Changes Which are Blocked by AB928



**Figure 7 (A)** Real-time PCR for adenosine receptor expression from NSCLC cell lines showing high A<sub>2b</sub>R expression. **(B)** NanoString data showing DE genes (fold change >1.5) driven by NECA (5 μM) in H650 NSCLC cells. **(C)** Comparison of dual vs A<sub>2a</sub>R selective antagonists in blocking NECA-stimulated gene expression changes in NSCLC cell lines.

## Conclusions

- In myeloid cells and A<sub>2b</sub>R-expressing cancer cell lines, dual A<sub>2a</sub>R/A<sub>2b</sub>R antagonism with AB928 prevents adenosine/NECA induced immuno-suppression and gene expression changes greater than A<sub>2a</sub>R-selective antagonism.
- These studies demonstrate an important role for A<sub>2b</sub>R in adenosine-mediated immunosuppression and provide a mechanistic rationale for stimulation of anti-tumor immune responses with the dual adenosine receptor antagonist AB928, which is currently being studied in several Phase 1b clinical trials.