Introduction
Adenosine, generated through the hydrolysis of extracellular adenosine monophosphate (AMP) by the ecto-5-nucleotidase CD73, is an important mechanism for immunosuppression in cancer development. Adenosine’s suppressive effects on immune cells are driven primarily through 2 of the 4 adenosine receptors, A2AR and A3R. We have previously shown that adenosine-mediated suppression of T cells can be blocked by the dual A2AR/A3R antagonist, AB928, which is currently being evaluated in several studies in cancer patients. Herein, we show that AB928 is capable of relieving adenosine-mediated immune suppression using human in vitro cell cultures, advanced gene expression studies, and mouse syngeneic tumor models.

Methods
The ability of AB928 to inhibit adenosine-mediated suppression of dendritic cell function in vivo was assessed using human monocyte-derived dendritic cells (moDC). Briefly, moDC were generated from freshly isolated CD14+ monocytes and differentiated with 10ng/mL CSF for 7 days +/- adenosine/ETNHA +/- AB928. Cells were then taken for NanoString analysis or placed in a mixed lymphocyte reaction (MLR) with CD4+ T cells. Mouse syngeneic tumor studies were conducted using C57BL/6 mice inoculated with mouse mammary tumor AT3-OVA or melanoma B16-F10 cells. Tumors were subsequently treated with doxorubicin or α-PD-1 +/- AB928.

TCGA Analysis of Human Tumors for CD73 and TNAP

CD73 is Expressed on Cancerous and Stromal Cells in Human Tumors

Figure 2. (A) Representative images of immunostaining for CD73 (brown) and CD8 (purple) on human FFPE tumor samples. Strong staining can be seen in both tumor (left) and stromal cells (right panel). (B) Quantification of IHC staining area from cores of tumor microarrays stained with CD73 (left panel). Strong correlation was seen with gene expression patterns from TCGA database (right) where each dot represents one tumor type: T, tumor; S, stroma.

AB928 Blocks Adenosine-Mediated Suppression of moDC Maturation

Adenosine Drives Large Gene Expression Changes in moDC

Figure 4. (A) Heat map of NanoString data displaying the gene expression levels of differentially expressed genes (fold change > 2.0 or < -2.0 and p value <0.05) from moDC before (left panel) and after (right panel) maturation with LPS. (B) Table showing the normalized gene expression counts, fold change, and p value of LPS matured moDC. moDC differentiated in the presence of adenosine show decreased DC maturation markers (top) and augmented expression of secreted molecules (bottom). (C) Heat map displaying differentially expressed genes in pre-maturation moDC differentiated with adenosine +/- AB928 (dual A2AR/A3R antagonist) or a selective A2AR antagonist. Heat map colors: Blue=low, blue=medium, yellow=high expression.

Peripheral Inhibition of Adenosine Receptor-mediated pCREB Correlates with Anti-Tumor Efficacy

Figure 5. (A) AB928 (30 or 100 mg/kg PO BID; dosing from day1) results in significantly decreased tumor growth (left panel) relative to vehicle **p<0.01. (B) AB928 inhibits adenosine receptor-mediated increases in pCREB in murine whole blood at trough.

Conclusions
• AB928 prevents adenosine-mediated gene expression changes and suppression of immune cell function in vitro
• AB928 combines with α-PD-1 or standard of care chemotherapy to suppress tumor growth in syngeneic mouse models
• AB928 is currently being tested in clinical trials in combination with either standard of care chemotherapy or α-PD-1 in tumor types that express high levels of CD73 and/or TNAP

AB928 in Combination with Doxorubicin Results in Greater AT3-OVA Tumor Control

AB928 Reduces B16-F10 Tumor Growth in Combination with anti-PD-1 Therapy

Figure 7. (A) AB928 (100 mg/kg PO BID) combined with doxorubicin results in significantly decreased tumor growth (left panel) and mass (right panel) relative to chemotherapy alone. ** p<0.01. (B) H&E staining of tumor sections shows AB928 + Dox (6 mg/kg) results in increased ECM and immune infiltrates compared to Vehicle + Dox.

Figure 6. (A) AB928 (100 mg/kg PO BID) combined with doxorubicin drives large gene expression changes with significantly decreased tumor growth (left panel) and mass (right panel) relative to chemotherapy alone. **p<0.01. (B) H&E staining of tumor sections shows AB928 + Dox (6 mg/kg) results in increased ECM and immune infiltrates compared to Vehicle + Dox.