Adenosinergic Axis Provides an Anchor in Multiple Anti-tumor Combination Strategies


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ABSTRACT

Tumor cell death induced by hypoxia or chemotherapy releases large amounts of ATP (adenosine triphosphate). ATP is rapidly converted to AMP (adenosine monophosphate) which, in turn, is converted by hypoxia-induced CD73 into adenosine (ADO). ADO suppresses immune responses, including those of T cells, NK cells and dendritic cells, through activation of adenosine receptors A2A-R and A2B-R. Exhausted T cells and NK cells express high levels of several immune checkpoint (IC) proteins, including PD-1 and TIGIT. Combined expression of IC proteins with CD73 or A2A-R/A2B-R in multiple tumor types presents an opportunity for novel combinatorial therapies. We present here in vitro and in vivo data on the utility of combining adenosinergic pathway inhibition with multiple therapeutic modalities, including antagonistic antibodies targeted against PD-1 and TIGIT, as well as platinum-based chemotherapeutics. Mechanistically, AMP abrogated the enhanced T cell activation and IFN-γ production mediated by blocking PD-1/PD-L1 and TIGIT, an effect that was reversed by CD73 inhibitors (CD73i). Global gene changes measured using NanoString® include down-regulation of activation markers, co-stimulatory molecules (CD28, ICOS, 4-1BB), and IC proteins (PD-1, TIM-3, LAG3), changes that were confirmed by flow cytometry. Thus, activation of the adenosinergic pathway may limit the efficacy of IC inhibitors and agonistic antibodies. In vivo proof of concept studies combining chemotherapy or α-PD-1/α-TGIT antagonistic antibody with CD73i or a dual A2A-R/A2B-R antagonist result in strong suppression of tumor growth as well as measurable changes in the immune compartment. These data provide a rationale for strategically combining adenosinergic pathway inhibitors with a range of clinically relevant interventions affecting a broad array of different immune cell types.

INTRODUCTION

Figure 1: Adenosine, a Potent Immune Suppressor, is Generated Through Sequential Hydrolysis of ATP by CD39 and CD73. Extracellular adenosine is removed from the microenvironment by adenosine deaminase (ADA) tethered to the cell surface by CD79. CD73 inhibitors used in this study include A000830, A001421, and AB680. AB928 is a dual A2A-R and A2B-R antagonist.

RESULTS

Figure 2: AB680 Limits the Inhibitory Effect of AMP on CD4+ T Cell Activation. Proliferation (A) and cytokine secretion (B) in the presence of AMP and varying concentrations of AB680 tested.

Figure 3: AB680 Reverses the Activity of AMP on α-PD-1 (AB122) and α-TGIT (AB154) Antibodies in MLR. AB122 (A) or AB122 and AB154 (B) were simultaneously added to CD4+ T cells and mononuclear DC cultures containing 100 μM AMP. Addition of AB680 completely reversed the inhibitory effects of AMP.

Figure 4: Opposing Effects of AMP and α-PD-L1 on Immune Modulatory Protein Expression in MLR. (A) Expression of indicated proteins in the presence of AMP (top row) or α-PD-L1 antibody (bottom row) compared to samples treated with isotype determined by flow cytometry. Each color represents one donor for a total of 4 donors tested. The reversibility of AMP-mediated immune checkpoint protein expression was shown using CD73 inhibitors (B). Gray bars represent AMP alone; green bars represent α-PD-L1 blocking antibody alone, red bars represent AMP + α-PD-L1 blocking antibody, and blue bars represent AMP + α-PD-L1 blocking antibody + CD73i. (C) Global transcriptional changes in MLR with AMP or with AMP + AB680. Data normalized to isotype controls were used for subsequent analysis. Numbers indicate fold change compared to AB122 alone.

Figure 5: AB928 Enhances the Activity of Oxaliplatin (Ox) in AT3.OVA Breast Cancer Model. Tumor volume changes in the presence of Ox alone or Ox + AB928 (A), and percentage of tumor antigen-specific CD8+ T cells (B) are shown. Pink circles indicate days when Ox dosing occurred.

Figure 6: CD73 Inhibitors Enhance the Activity of α-PD-1 in B16.F10 Melanoma Model. In-house generated CD73 inhibitor, A000830 or AB680, together with α-PD-1 antibody (RMP-1-14) were dosed when tumors reached a volume of 50 mm3. Change in tumor volume (A) associated with changes in the immune cell infiltrates (B) were similar. Similar to A000830, AB680 also increased efficacy of α-PD-1/α-TGIT antibody as measured by tumor volume (C) and survival curves (D). Mice bearing tumors > 1000 mm3 were sacrificed as per protocol. Pink circles indicate days when α-PD-1 antibody dosing occurred.

METHODS

Human CD4+ T cells and CD8+ monocytes were isolated from buffy coats using various RosetteSep and EasySep Enrichment cocktails. The ability of AB680 to rescue AMP-mediated inhibition of T cell activation was evaluated using CD3/CD28/CD2 stimulation. Mixed lymphocyte reactions (MLRs) were established by adding GM-CSF and IL-4 differentiated mononuclear DCs with allogeneic CD4+ T cells. In some experiments, α-PD-1 (AB122) and α-TGIT (AB154) blocking antibodies were added. Alternatively, α-PD-1 blocking antibody was used instead of AB122. RNA from DC MLR cultures was isolated and gene expression changes identified using nCounter® Inflammation v2 (NanoString Technologies). AT3.OVA and B16.F10 cells were implanted into the flank of 6-week old C57BL/6J. 5 mg/kg oxaliplatin and AB928 were dosed starting at 50 mm3 tumor volume. 2.5 mg/kg α-PD-1 (IP Q3D) or isotype control antibody (2A3) and A000830 or AB680 were dosed when tumor volume reached 50 mm3.

SUMMARY

❖ AMP is a potent inhibitor of T cell activation even in the presence of PD-1/PD-L1 blockade
❖ Exogenous AMP up-regulates gene expression of immune suppressive genes, including IL-10, TGF-β, IDO-1, PD-L1, PD-L2, and various immune checkpoint proteins
❖ Activation of adenosine receptors in human and mouse T cells results in quantifiable changes in immune checkpoint protein expression that can be reversed by a dual A2A-R/A2B-R antagonist or CD73 inhibitors