AACR
Annual Meeting, April 5th-10th, 2024
Abstract 2478

Inhibition of CD39 by AB598 Increases Extracellular ATP Resulting in Activation of Myeloid Cells and T Cells to Enhance Anti-Tumor Immunity

Ke Jin, Julie Clor, Kaustubh Parashar, Rebecca Fuchs, LC Stetson, Sean Cho, Matthew J. Walters, Ester Fernández-Salas, Christine E. Bowman

Arcus Biosciences, Inc.; 3928 Point Eden Way, Hayward, CA 94545 (USA)

Introduction

- CD39 (*ENTPD1*), an ecto-ATPase, catalyzes the successive hydrolysis of ATP into AMP, resulting in decreased availability of immunostimulatory ATP in the tumor microenvironment. CD39 inhibition leads to increased levels of ATP, promoting anti-tumor responses through the activation of immune cells.
- AB598 is a novel, humanized, Fc-silent (FcS) anti-CD39 therapeutic antibody that potently binds to CD39 and inhibits its enzymatic activity with sub-nanomolar potency. AB598 is currently being investigated in a Phase 1 clinical trial (NCT05891171).
- Here we present an *in vitro* triple cell co-culture system, demonstrating that extracellular ATP can be released from cancer cells treated with immunogenic cell death (ICD)-inducing chemotherapy. AB598 preserves released extracellular ATP to directly activate myeloid cells, leading to increased T cell functionality.

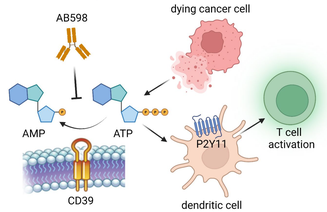
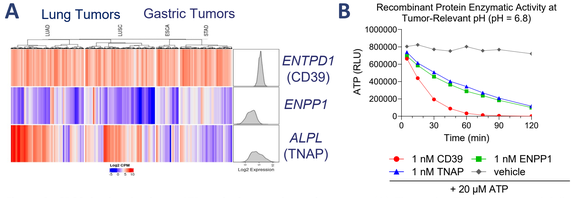


Figure 1. CD39 inhibition promotes anti-tumor immunity. Extracellular ATP is released when cancer cells are killed by immunogenic cell death (ICD)-inducing agents. Extracellular ATP is degraded by CD39 rapidly. CD39 enzymatic inhibition by AB598 sustains a high level of extracellular ATP within solid tumors resulting in myeloid cell activation. Cell-type illustrations generated with Biorender.com

Results

CD39 is the Dominant ATPase in the Tumor Microenvironment



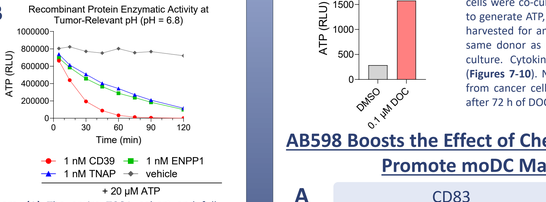


Figure 2. CD39 is the dominant ATPase in lung and gastric tumors. (A) The entire TCGA cohort and full transcriptome were TMM (Trimmed Mean of M-Values) normalized and the log2 CPM values of selected genes are represented on the heatmap. The expression distribution is also depicted in the histograms to the right. **(B)** The recombinant protein of each ATPase was incubated with 20 µM ATP and the remaining ATP was measured at different time points using Kinase-Glo.

AB598 Inhibits CD39 Enzymatic Activity in Dissociated Tumor Cells

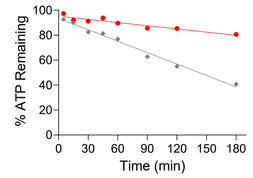


Figure 3. Anti-CD39 treatment reduces ATP degradation in human gastric tumor samples. Dissociated cells from gastric tumors were incubated with 100 nM AB598 or isotype control (IgG1 FcS). 20 µM ATP was added to cells and the remaining ATP was measured over a period of 3 hours (h). The same experiment was performed with an additional donor sample and similar results were achieved (data not shown).

ATP, Preserved by AB598, Activates moDCs through P2Y11

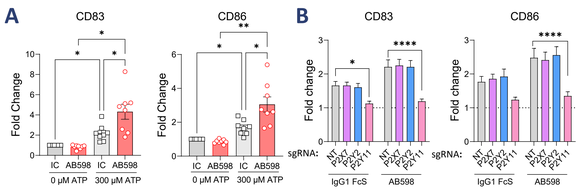


Figure 4. AB598 enhanced moDC activation in the presence of ATP is mediated by P2Y11 receptors. (A-B) moDCs were treated with AB598 or IgG1 FcS (IC in figure) before the addition of ATP. CD83 and CD86 were assessed by flow cytometry. Data was normalized to the 0 µM ATP isotype control-treated condition on a per donor basis. N = 6 - 8. **(B)** CRISPR knockout of the indicated gene or a non-targeting (NT) guide showed that the effect of ATP + AB598 on moDCs is mediated by P2Y11. Statistical significance was calculated with a one-way ANOVA **(A)** or a two-way ANOVA **(B).**

Triple Cell Co-Culture System for Myeloid-Driven T Cell Activation

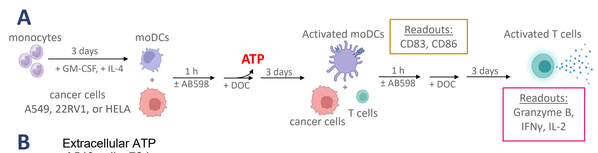


Figure 5. Protocol of triple cell co-culture system. (A) The co-culture approach is employed in **Figures 6-10**. moDCs were differentiated from monocytes. Cancer cells were co-cultured with moDCs in the presence of chemotherapy treatment to generate ATP, load tumor-specific antigens, and activate moDCs. moDCs were harvested for analysis (**Figure 6**). Alternatively, T (CD4+ or CD8+) cells from the same donor as moDCs, to avoid allogeneic activation, were added to the co-culture. Cytokine production was measured to determine T cell activation (**Figures 7-10**). No exogenous ATP was added to the system. ATP was released from cancer cells with docetaxel (DOC) treatment. **(B)** Enhanced eATP release after 72 h of DOC treatment in A549 lung cancer cells.

AB598 Boosts the Effect of Chemotherapy-Driven ATP Release to Promote moDC Maturation and Activation

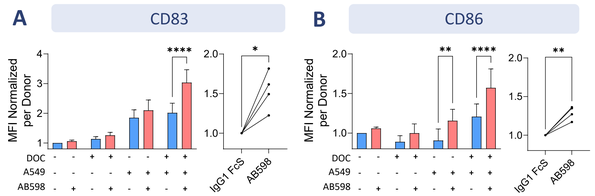


Figure 6. AB598 enhances moDC maturation and activation in the presence of chemotherapy-triggered ATP release. Co-culture assay was performed as shown in Figure 5 through the moDC step. In the bar graphs, data was normalized to the moDC only group on a per donor basis and statistical significance was determined with a two-way ANOVA. The effect of AB598 in the full co-culture system is shown in the line graphs to right, re-normalized to the IgG1 FcS-treated data on a per donor basis. Significance was determined through paired t-test.

Statistics Summary. In the entire poster, N represents the number of unique human donors, bar heights are the mean, error bars are the SEM, and *P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001.

AB598 Boosts the Effect of Chemotherapy to Promote moDC Activation to Enhance T Cell Function

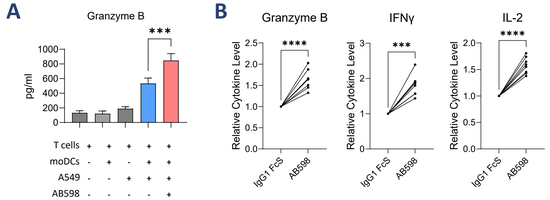


Figure 7. Chemotherapy-generated ATP, preserved by AB598, results in increased T cell functionality. (A-B) Co-culture assay was performed as shown in Figure 5 with CD8+ T cells. **(A)** Granzyme B concentration in the cell culture supernatant was measured and statistical significance was determined with a one-way ANOVA. **(B)** Granzyme B, IFNγ, and IL-2 production were measured as indications of CD8+ T cell activation. Cytokine concentrations were normalized to the IgG1 FcS-treated data on a per donor basis. Data show AB598 treatment significantly increased the production of all three cytokines. Significance was determined through paired t-test. AB598 also significantly enhances cytokine production from CD4+ T cells (data not shown).

AB598 Enhances APC-Dependent T Cell Activation via Elevated ATP Rather Than Relief of Adenosine-Mediated Immune Suppression

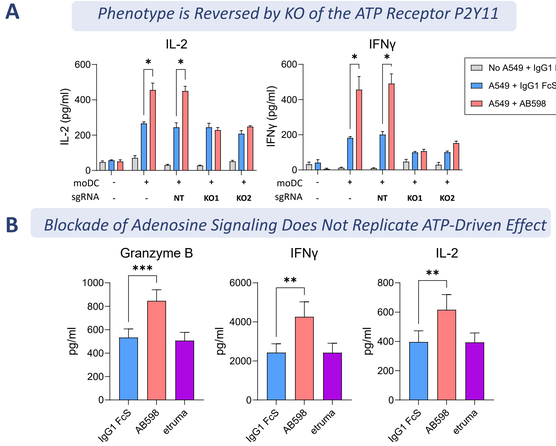


Figure 8. Increase in granzyme B, IFNγ, and IL-2 production with AB598 treatment is driven by ATP. Co-culture assay was performed as shown in Figure 5 with CD4+ **(A)** or CD8+ **(B)** T cells. **(A)** CRISPR knockout of P2Y11 in moDCs with two different sgRNAs (KO in legend) or a non-targeting (NT) sgRNA was performed. Both IL-2 and IFNγ production showed AB598 lost the ability to enhance T cell activation when the ATP-dependent purinergic receptor P2Y11 was KO in moDCs, indicating that the enhanced T cell function is mediated by moDC P2Y11. N = 4. **(B)** Granzyme B, IFNγ, and IL-2 production were measured as indications of CD8+ T cell activation. Data show that AB598 treatment significantly increased the production of all three cytokines. However, etruma, a dual $A_{2A}R/A_{2B}R$ receptor antagonist didn't affect CD8+ T cell activation, indicating that the effect is driven by increased ATP, not decreased adenosine. Statistical significance was calculated with a two-way ANOVA **(A)** or a one-way ANOVA **(B)**.

Contacts: Ke Jin, PhD, kjin@arcusbio.com and Christine Bowman, PhD, cbowman@arcusbio.com

In the Presence of ADO-Induced Immunosuppression, Combining AB598 + Etruma Results in More Effective T Cell Activation

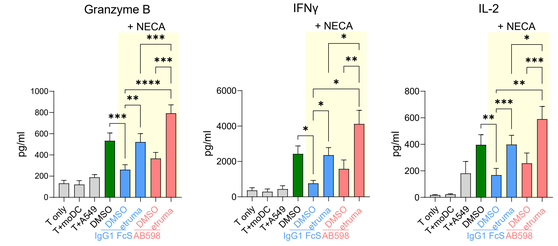


Figure 9. The combination of AB598 and etruma improves cytokine production from CD8+ T cells in the presence of NECA, an adenosine (ADO) receptor agonist. Co-culture assay was performed as shown in **Figure 5** using CD8+ T cells. Granzyme B, IFNγ, and IL-2 production indicate that NECA inhibits CD8+ T cell activation. Etruma abolished the inhibitory effect of NECA and resulted in better activation of CD8+ T cells when combined with AB598. N = 8. Statistical significance was calculated with a one-way ANOVA. Colored bars contain all three cell types (T cells, moDCs, A549) with DOC treatment and the yellow shaded area indicates conditions with NECA added. Blue bars indicate treatment with isotype control and pink bars indicate treatment with AB598.

CD39 Is Expressed on Tumor-Infiltrating Immune Cells

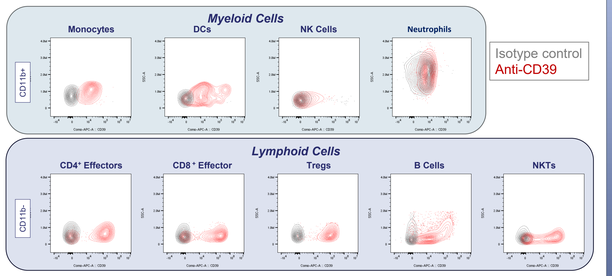


Figure 10. CD39 expression profiling in human gastric tumor-infiltrating immune cells. Flow cytometry analysis showing the distribution of CD39 across myeloid and lymphoid populations from a representative dissociated gastric tumor sample. Immune cell subpopulations are defined as monocytes (HLA-DR+CD14+CD16-), DC (HLA-DR+CD11c+), NK (CD3-CD56+), Neutrophils (FSC/SSC, CD16+), CD4+ effector (CD3+CD4+CD45RA-CCR7-), CD8+ effector (CD3+CD8+CD45RA-CCR7-), Tregs (CD3+CD8-CD56-FOXP3+), B (CD3-CD19+), and NKT (CD3+CD56+) cells.

Conclusions

- AB598 potently inhibits CD39, the dominant ATPase mediating ATP degradation in the tumor microenvironment.
- Treatment of cancer cells with chemotherapy induces ATP release, which when preserved by AB598, results in increased antigen presenting capability in moDCs, which in turn promotes CD8+ T cell activation.
- This effect is driven by ATP stimulation of moDCs, not a relief of adenosine-mediated immunosuppression on T cells, demonstrated by the loss of both the myeloid and T cell activation phenotype with a myeloid-cell specific P2Y11 knockout or treatment of the entire co-culture system with etruma, which blocks adenosine signaling.
- In the presence of adenosine-mediated immunosuppression, the combination of AB598 and etruma allows for enhanced T cell activation compared to either agent alone.