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Abstract 2154

Inhibition of CD39 by AB598 Enhances the Effects of Chemotherapy and anti-PD-1 Therapy to Promote Myeloid Cell and T Cell anti-Tumor Immunity

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Background

- The inhibition of CD39 (*ENTPD1*) halts the degradation of extracellular ATP (eATP), a potent immunostimulatory signal in the tumor microenvironment (TME), leading to an immunostimulatory environment resulting in anti-tumor immunity.
- AB598 is a novel, humanized, Fc-silent (FcS) anti-CD39 monoclonal antibody that potently binds to CD39 and inhibits its enzymatic activity with sub-nanomolar potency. AB598 is currently being investigated in ARC-25, a Phase 1 clinical trial (NCT05891171).
- Here we present *in vitro* cell co-culture systems, demonstrating that AB598 preserves eATP, exogenously added or endogenously generated by immunogenic cell death (ICD), directly activates macrophages and dendritic cells, to enhance NK and T cell functionality.

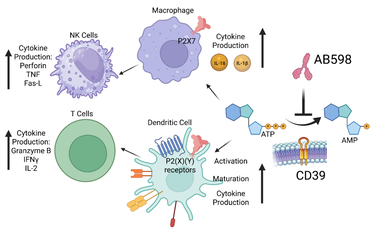

Figure 1. CD39 inhibition promotes anti-tumor immunity. Extracellular ATP (eATP) is released when cancer cells are killed by immunogenic cell death (ICD)-inducing agents. Extracellular ATP is rapidly degraded by CD39. CD39 enzymatic inhibition by AB598 sustains a high level of eATP within solid tumors resulting in macrophages and dendritic cells activation, to enhance NK and T cell functionality.
Cell type illustrations generated with BioRender.com

Results

CD39 is Highly Expressed in the Tumor Microenvironment

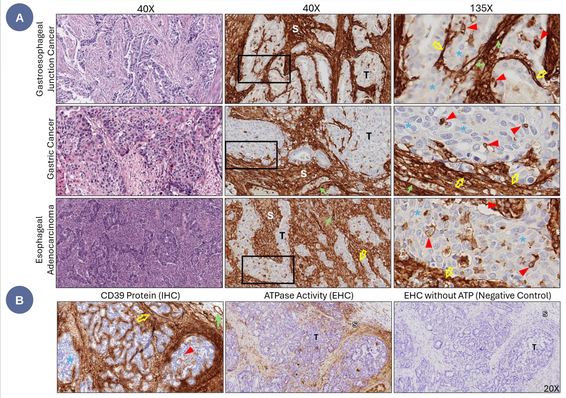

Figure 2. CD39 protein expression and enzymatic activity are enriched in non-cancer cells in the TME. (**A**) Immunohistochemistry (IHC) for CD39 shows high expression levels in stromal fibroblasts (yellow open arrows), endothelial cells (green arrows), and tumor-associated immune cells morphologically consistent with lymphoid and myeloid lineages (red arrowheads), while cancer cells are negative (blue asterisks). (**B**) Enzyme histochemistry (EHC) to detect deposition of free phosphate as a result of ATPase hydrolysis was performed on fresh frozen tumor tissue in the presence of exogenous ATP and shows CD39 enzyme activity correlates spatially with protein expression.

CD39 is Highly Expressed in Endothelial and Fibroblasts/Smooth-Muscle Cell Populations in Human Gastric Tumors

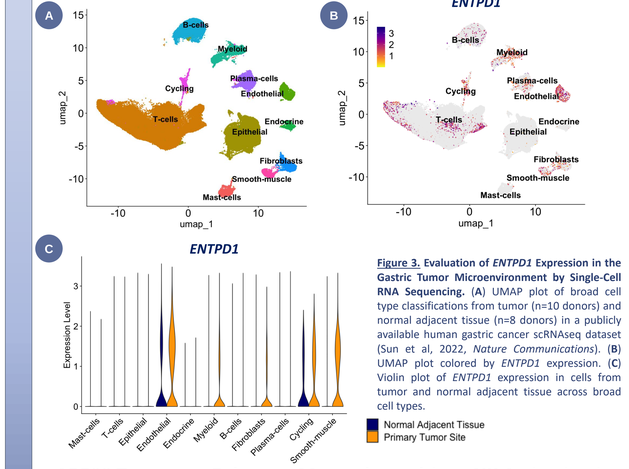

Figure 3. Evaluation of *ENTPD1* Expression in the Gastric Tumor Microenvironment by Single-Cell RNA Sequencing. (**A**) UMAP plot of broad cell type classifications from tumor (n=10 donors) and normal adjacent tissue (n=8 donors) in a publicly available human gastric cancer scRNAseq dataset (Sun et al, 2022, *Nature Communications*). (**B**) UMAP plot colored by *ENTPD1* expression. (**C**) Violin plot of *ENTPD1* expression in cells from tumor and normal adjacent tissue across broad cell types.

AB598 Treatment Enhances Macrophage-driven NK Activation in the Presence of ATP

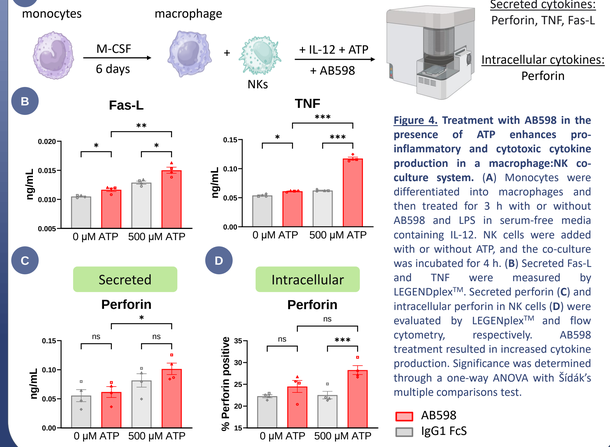

Figure 4. Treatment with AB598 in the presence of ATP enhances pro-inflammatory and cytotoxic cytokine production in a macrophage:NK co-culture system. (**A**) Monocytes were differentiated into macrophages and then treated for 3 h with or without AB598 and LPS in serum-free media containing IL-12. NK cells were added with or without ATP, and the co-culture was incubated for 4 h. (**B**) Secreted Fas-L and TNF were measured by LEGENDplex™. Secreted perforin (**C**) and intracellular perforin in NK cells (**D**) were evaluated by LEGENplex™ and flow cytometry, respectively. AB598 treatment resulted in increased cytokine production. Significance was determined through a one-way ANOVA with Šídák's multiple comparisons test.

Triple Cell Coculture System Recapitulates Chemotherapy Treatment in Gastric Tumors

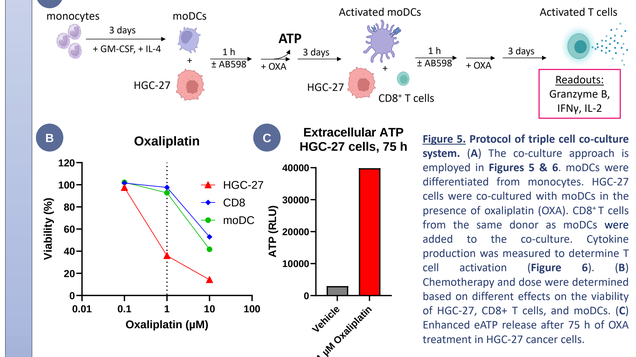

Figure 5. Protocol of triple cell co-culture system. (**A**) The co-culture approach is employed in **Figures 5 & 6**. moDCs were differentiated from monocytes. HGC-27 cells were co-cultured with moDCs in the presence of oxaliplatin (OXA). $CD8^+$ T cells from the same donor as moDCs were added to the co-culture. Cytokine production was measured to determine T cell activation (**Figure 6**). (**B**) Chemotherapy and dose were determined based on different effects on the viability of HGC-27, CD8+ T cells, and moDCs. (**C**) Enhanced eATP release after 75 h of OXA treatment in HGC-27 cancer cells.

AB598 Boosts the Effect of Chemotherapy to Promote moDC Activation Resulting in Enhanced T Cell Function

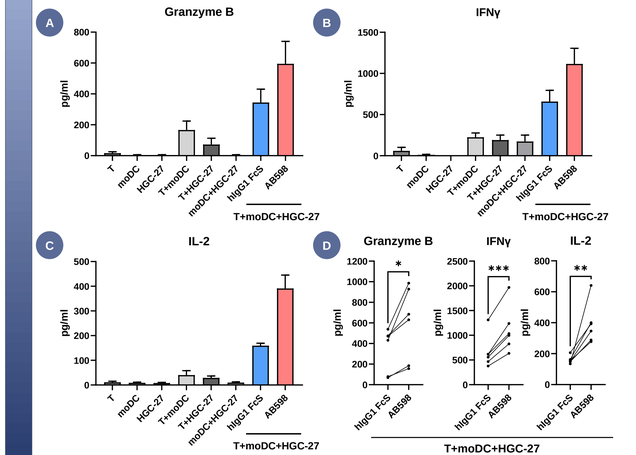

Figure 6. Chemotherapy-generated ATP, preserved by AB598, results in increased T cell functionality. Co-culture assay was performed as shown in **Figure 5**. (**A**) Granzyme B, (**B**) IFNγ, and (**C**) IL-2 concentration in the cell culture supernatant was measured. Cytokine production was used as indicator of $CD8^+$ T cell activation. (**D**) Cytokine concentrations were normalized to the IgG1 FcS-treated data on a per donor basis. Data show AB598 treatment significantly increased the secretion of all three cytokines. Significance was determined through paired t-test.

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Poster available at http://arcusbio.com/our-science/publications/

The Combination of Anti-PD-1 and Anti-CD39 Antibodies Leads to Myeloid Cell Activation and Tumor Control

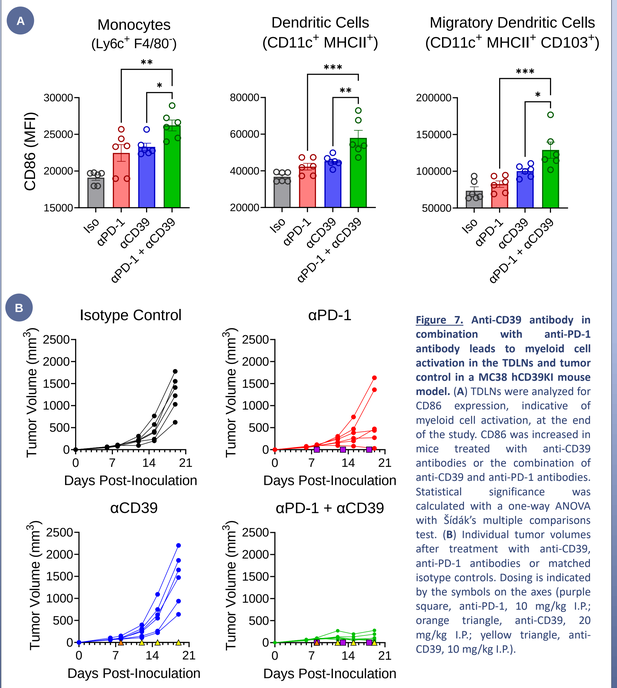

Figure 7. Anti-CD39 antibody in combination with anti-PD-1 antibody leads to myeloid cell activation in the TDLNs and tumor control in a MC38 hCD39KI mouse model. (**A**) TDLNs were analyzed for CD86 expression, indicative of myeloid cell activation, at the end of the study. CD86 was increased in mice treated with anti-CD39 antibodies or the combination of anti-CD39 and anti-PD-1 antibodies. Statistical significance was calculated with a one-way ANOVA with Šídák's multiple comparisons test. (**B**) Individual tumor volumes after treatment with anti-CD39, anti-PD-1 antibodies or matched isotype controls. Dosing is indicated by the symbols on the axes (purple square, anti-PD-1, 10 mg/kg I.P.; orange triangle, anti-CD39, 20 mg/kg I.P.; yellow triangle, anti-CD39, 10 mg/kg I.P.).

Conclusions

- CD39 is highly expressed in cells in the tumor stroma, including endothelial cells, cancer associated fibroblasts, smooth-muscle cells, and immune cells.
- ATP, which is preserved by AB598, results in macrophage activation that in turn promotes NK cell activation.
- Treatment of gastric cancer cells with ICD-inducing chemotherapy leads to ATP release, which is preserved by AB598, resulting in increased moDC maturation that promotes $CD8^+$ T cell activation.
- The combination of anti-CD39 and anti-PD-1 antibody treatments leads to myeloid cell activation in TDLNs and tumor control in the MC38 hCD39KI mouse model.

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