# AB598, a CD39 Inhibitory Antibody, Promotes Immune-Mediated Tumor Control

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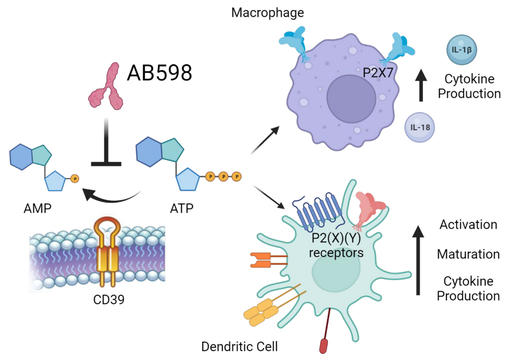
## Background

- AB598 inhibits the enzymatic activity of CD39, the dominant ATPase in the tumor microenvironment (TME). CD39 inhibition allows ATP levels to rise locally, leading to the activation of myeloid cells, allowing them to activate T cells and enhance control of tumor growth.
- AB598 is potent and specific, inhibiting human CD39 with sub-nanomolar potency. It effectively inhibits human CD39, but it does not inhibit murine CD39, posing a challenge for studying CD39 inhibition in immune-competent syngeneic tumor models.
- Here we present data from an *in vitro* co-culture system, a MOLP8 xenograft model, and a human CD39 knock-in (hCD39KI) mouse model that highlight the ability of AB598 to inhibit enzymatic activity to activate the immune system to drive an anti-tumor immune response.

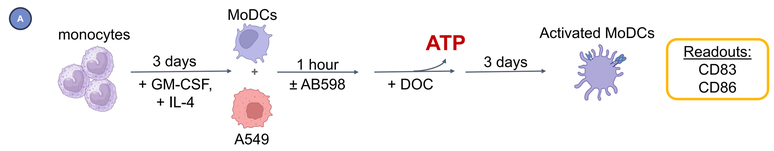



*AB598 is a highly potent and specific IgG1 Fc-silent antibody targeting CD39*

**Figure 1. CD39 inhibition promotes anti-tumor immunity by myeloid cell activation.** CD39 suppresses the immune system by degrading immunostimulatory extracellular ATP (eATP) released in the TME by dying cells. Inhibiting CD39 can increase levels of eATP resulting in myeloid cell activation and release of pro-inflammatory cytokines. Schematic created with BioRender.com.

## Results

### AB598 Boosts the Effect of Chemotherapy to Promote MoDC Activation



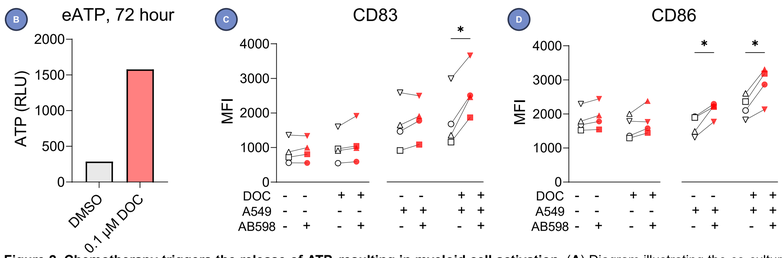




**Figure 2. Chemotherapy triggers the release of ATP, resulting in myeloid cell activation.** (**A**) Diagram illustrating the co-culture approach employed in (**B-D**). (**B**) Enhanced eATP release observed after 72 h of docetaxel (DOC) treatment in A549 lung cancer cells. (**C**) Increased CD83 and (**D**) CD86 protein expression in moDCs when co-cultured with A549 cells and DOC in the presence of AB598. Significance was determined through a two-way ANOVA using Tukey's multiple comparisons test with the Geisser-Greenhouse correction, *P ≤ 0.05. Cell type illustrations generated with Biorender.com.

### AB598 Promotes ATP-Dependent Activation of *in vitro*-Derived Dendritic Cells and Macrophages

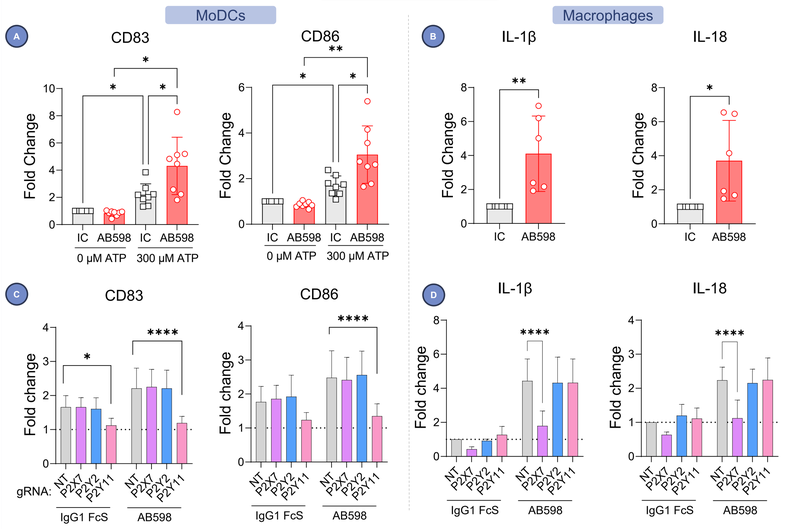
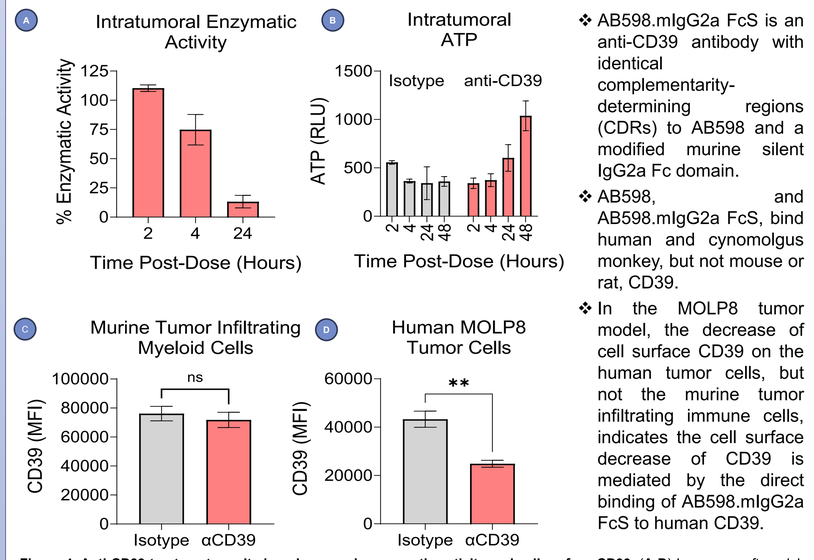



**Figure 3. AB598 enhances myeloid cell activation in the presence of ATP, mediated by P2X7 and P2Y11 receptors.** Monocytes were differentiated into moDCs (**A, C**) or macrophages (**B, D**). (**A, C**) MoDCs were treated with 100 nM AB598 or an isotype control (abbreviated IC or IgG1 FcS in figure) for 1 h before the addition of 0 or 300 μM ATP. CD83 and CD86 were assessed by flow cytometry 18 h after ATP addition. Data was normalized to the 0 μM ATP isotype control treated condition on a per donor basis, N = 6 - 8. (**B, D**) Macrophages were treated with 100 nM AB598 or an isotype control for 1 h, followed by 1 ng/mL LPS for 3 h, and then 500 μM ATP for 4 h. IL-1β and IL-18 measured from the supernatants indicated activation of the inflammasome. Data was normalized to the 500 μM ATP isotype control treated condition on a per donor basis, N = 8. (**C, D**) CRISPR knockout of the indicated gene and treatment with a non-targeting (NT) guide showed that the effect of ATP + AB598 on moDCs is mediated by P2Y11 and the effect of ATP + AB598 on macrophages is mediated by P2X7. Statistical significance was calculated with a one-way ANOVA with Tukey's multiple comparisons test (**A**), an unpaired t test (**B**), or a two-way ANOVA with Dunnett's multiple comparisons test (**C, D**). *P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001, **** P ≤ 0.0001. Error bars represent the standard deviation.

## Results

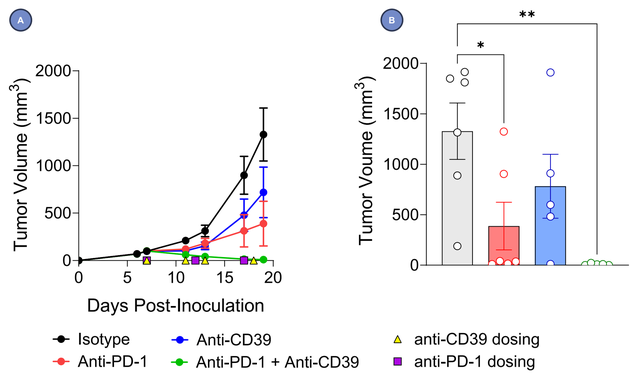
### Systemic AB598 Treatment Inhibits Intratumoral CD39 Enzymatic Activity, Elevates Intratumoral ATP, and Decreases Intratumoral CD39 in MOLP8 Tumors



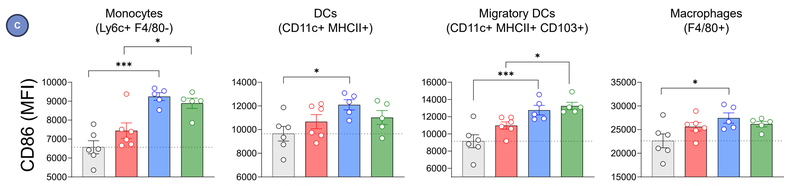

- AB598.mIgG2a FcS is an anti-CD39 antibody with identical complementarity-determining regions (CDRs) to AB598 and a modified murine silent IgG2a Fc domain.
- AB598, and AB598.mIgG2a FcS, bind human and cynomolgus monkey, but not mouse or rat, CD39.
- In the MOLP8 tumor model, the decrease of cell surface CD39 on the human tumor cells, but not the murine tumor infiltrating immune cells, indicates the cell surface decrease of CD39 is mediated by the direct binding of AB598.mIgG2a FcS to human CD39.

**Figure 4. Anti-CD39 treatment results in a decrease in enzymatic activity and cell surface CD39.** (**A-D**) In a xenograft model, severe combined immunodeficiency (SCID) mice were inoculated with human MOLP8 multiple myeloma tumors. When tumors reached 300-500 mm$^3$, a single intravenous administration of either AB598.mIgG2a FcS or a corresponding isotype control antibody was given. After 48 h, unmatched tumors were collected and evaluated for intratumoral enzymatic activity (**A**) or ATP (**B**). After 72 h, extracellular CD39 levels were assessed on both the murine tumor infiltrating immune cells, identified as CD11b$^+$ (**C**) and the human tumor cells, identified as CD47$^+$ (**D**). The height of the bars represents the mean, with N = 2 - 4, and the error bars depict the SEM. Statistical significance was determined using unpaired t-tests, with **P ≤ 0.01, ns = non-significant.

### Combination Treatment with Anti-CD39 and Anti-PD-1 Results in Greater MC38 Tumor Control




### Increased Myeloid Cell Activation in the Tumor Draining Lymph Nodes (TDLNs) with Anti-CD39 Treatment




*Minimal changes were seen in CD86 percent positivity on these cell types (data not shown), suggesting anti-CD39 treatment is driving a further activation of the myeloid cells already in the tumor microenvironment.*

### Decreased Cell Surface CD39 on Immune Cells in TDLNs with Anti-CD39 Treatment

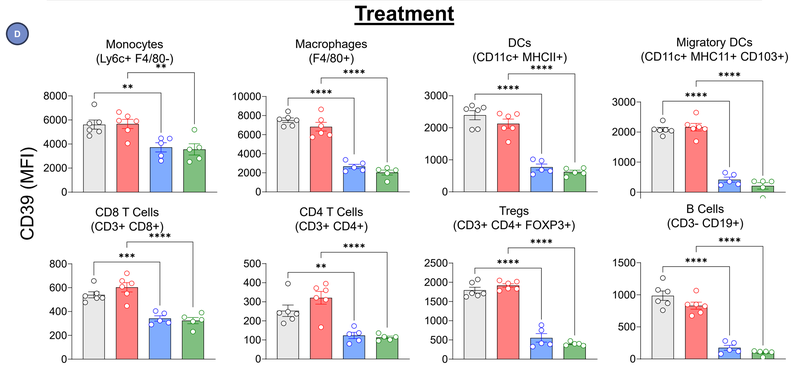
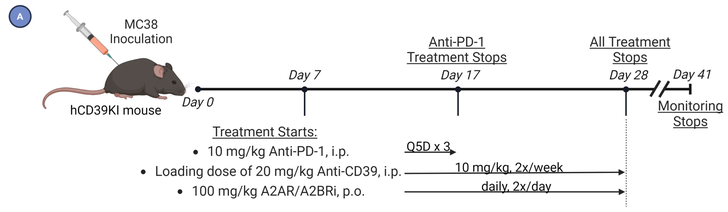
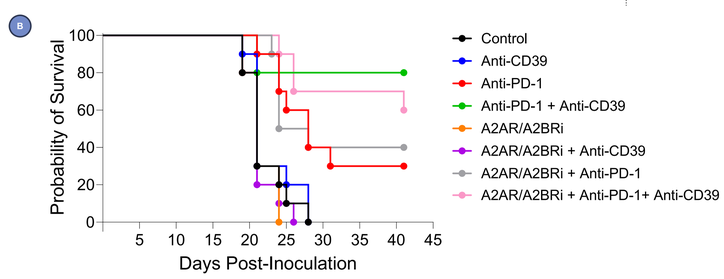



**Figure 5. Inhibition of CD39 leads to enhanced myeloid activation and a reduction in cell surface CD39 expression.** HCD39KI mice from Biocytogen were injected with 1E6 MC38 cells. When tumors reached approximately 80 mm$^3$, mice were treated as described with matched isotype controls in each condition (not noted in legend) (**A**). (**B**) Comparison of final tumor volumes. (**C, D**) Myeloid cells, including monocytes, dendritic cells (DCs), and macrophages, displayed significant increases in activation, most notably by CD86 MFI (**C**). A decrease in cell surface CD39 was observed in both myeloid and lymphoid populations in animals treated with anti-CD39 alone or in combination with anti-PD-1 (**D**). Statistical analysis was conducted using a one-way ANOVA (Dunnett's multiple comparison test, with a single pooled variance), with * indicating P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001, and **** P ≤ 0.0001. Error bars represent the standard deviation, with each symbol representing an individual animal within the group.

## Results

### Anti-CD39 Treatment Boosts Anti-PD-1 Efficacy in MC38 Tumors



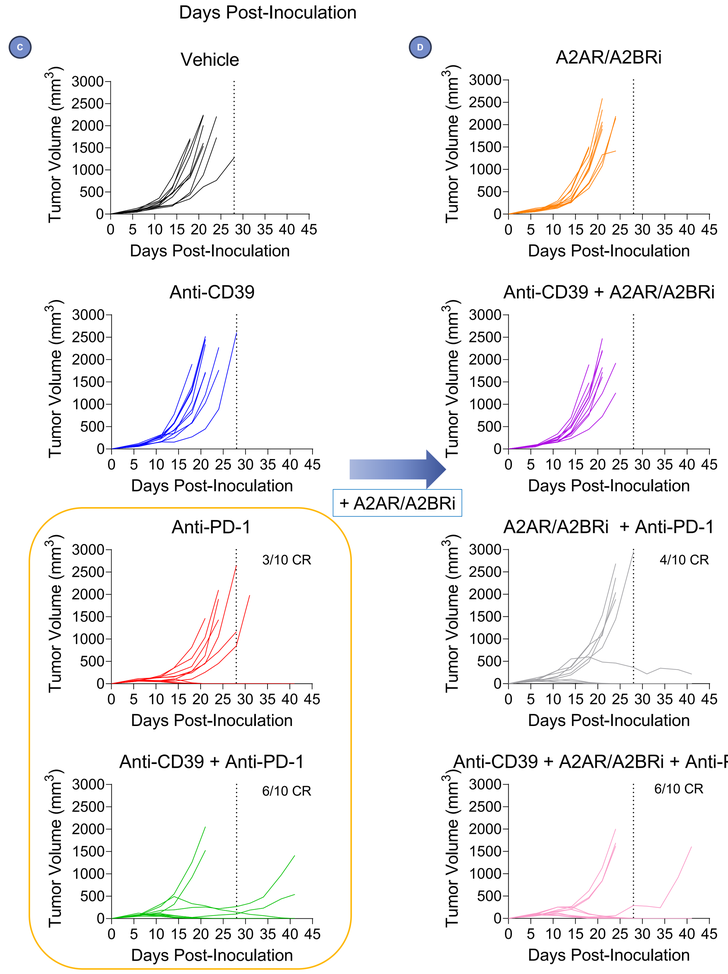




**Figure 6. Anti-CD39 in combination with anti-PD-1 is effective in a MC38 hCD39KI mouse model.** (**A**) Schematic illustrating the experimental set-up. Matched isotype controls, for anti-CD39 and anti-PD-1 and matched vehicle control, for A2AR/A2BRi were administered to groups not receiving the specified treatment. (**B**) Kaplan-Meier survival curves showing the probability of survival after inoculation with MC38 tumor cells and specified treatments. (**C, D**) Individual tumor growth curves without (**C**) or with (**D**) the addition of an A2AR/A2BR dual receptor antagonist. The dotted vertical line indicates the end of treatment. As highlighted in the graphs outlined in yellow, the combination of anti-CD39 and anti-PD-1 enhances efficacy. The addition of an A2AR/A2BR dual receptor antagonist did not lead to improved tumor control, suggesting the observed efficacy from CD39 inhibition is not driven by adenosine in this model. CR = complete remission.

## Summary

- AB598 boosts the effect of chemotherapy to activate myeloid cells, an effect that is enhanced in the presence of ATP and mediated through the P2Y11 and P2X7 receptors.
- Intratumoral CD39 enzymatic inhibition and resulting increased intratumoral ATP were observed after systemic AB598.mIgG2a FcS administration in a MOLP8 xenograft model.
- Enhanced tumor control was observed when anti-CD39 treatment was used in combination with anti-PD-1 treatment in the hCD39KI mouse model. This combination led to an increase in myeloid activation and a decrease in cell surface CD39 on immune cells in the TDLNs.
- The decrease in cell surface CD39 driven by AB598 is typically 50 - 90% on immune cells, emphasizing the primary mechanism of action of AB598 as a potent CD39 enzymatic inhibitor, opposed to an agent designed to eliminate CD39 from the TME.
- The ability of AB598.mIgG2a FcS to promote tumor control in combination with anti-PD-1 is driven by an additional factor, presumably ATP, rather than solely the relief of adenosine-mediated immunosuppression in the MC38 tumor model.

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