# Rationale for the Combination of AB598, a CD39 Blocking Antibody, and Chemotherapy for the Treatment of Solid Tumors

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## INTRODUCTION

- AB598 is a novel humanized monoclonal antibody under development by Arcus Biosciences as a solid tumor immuno-therapy targeting the enzymatic activity of CD39 (*ENTPD1*).
- CD39 is widely expressed in the tumor microenvironment, where inhibition of CD39 enzymatic activity promotes anti-tumor immune responses by increasing the immunostimulatory substrate ATP and decreasing the formation of the product AMP, a precursor to immunoinhibitory adenosine.
- Anti-CD39 treatment can be combined with immunogenic chemotherapy to increase intratumoral levels of ATP and further enhance immune responses. The data presented herein show that AB598 can stimulate myeloid cells to promote anti-tumor immunity.

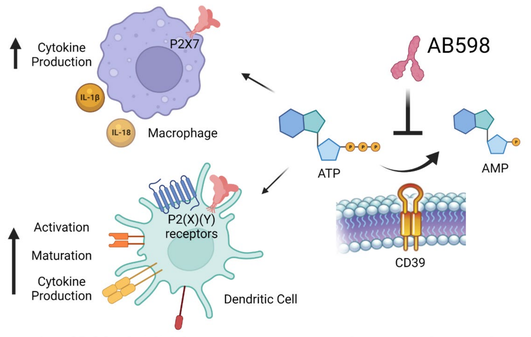



**Figure 1. CD39 inhibition promotes anti-tumor immunity by activating myeloid cells.** Schematic created with BioRender.com.

## RESULTS

### AB598 Potently Binds Cell Surface CD39

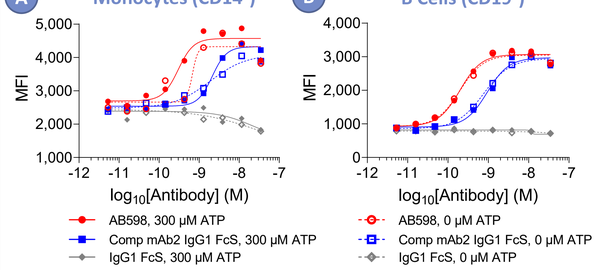



**Figure 2. Binding of AB598 to cell surface CD39.** Human PBMCs were used to determine binding of AB598 to specific cell types. Data are representative of 6 donors. The binding of AB598 is not affected by the presence or absence of ATP and AB598 binds with greater affinity than competitor antibody Comp mAb2 IgG1 FcS*.

### AB598 Treatment Bolsters Extracellular ATP in Combination With Oxaliplatin

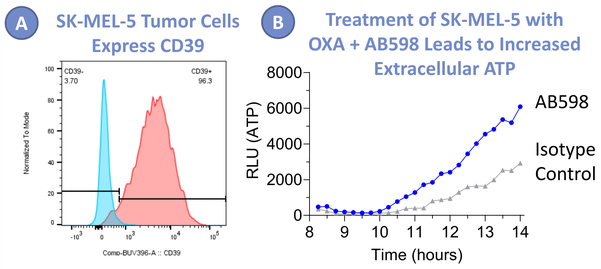



**Figure 3. Oxaliplatin (OXA) induces ATP release in cancer cell line.** SK-MEL-5 cells were treated with 250 µM OXA and 100 nM AB598 or a matched Fc-silent IgG1 isotype control for 8 hours. Extracellular ATP levels were measured over the following 6 hours using Promega RealTime Glo Extracellular ATP assay. Treatment of SK-MEL-5 cells with AB598 increased the amount of extracellular ATP in the cell culture supernatant as compared to the isotype control treated cells. AB598 treatment did not affect SK-MEL-5 cell viability (data not shown).

*Comp mAb2 IgG1 FcS is a competitor humanized antibody made using sequence information from patent filings.
**AB598 and Arcus anti-CD39 IgG4 share the same heavy and light chain variable domains.
FcS; Fc-silent.

### AB598 Promotes ATP-Dependent Dendritic Cell Maturation and Macrophage Inflammasome Activation

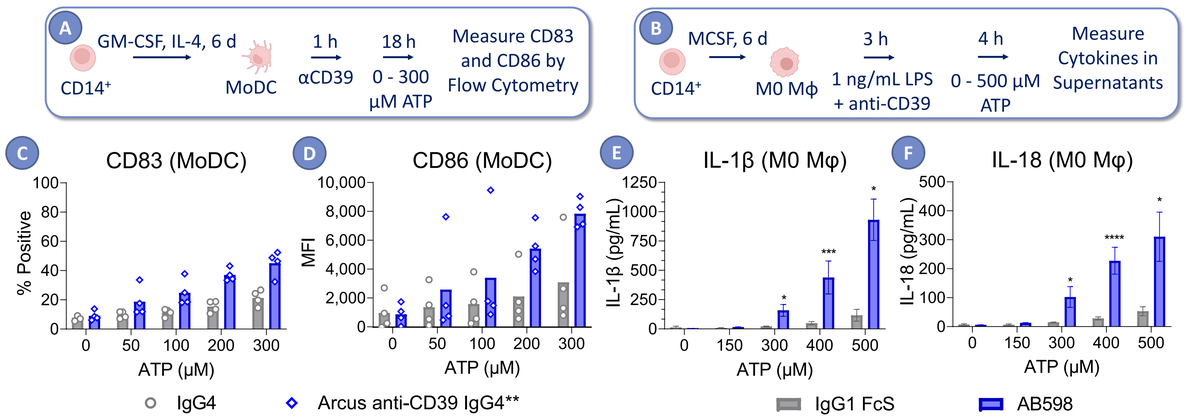



**Figure 4. A, C, D. Increased moDC maturation by AB598 treatment in the presence of ATP.** CD14 positively selected cells from peripheral blood were differentiated to generate monocyte-derived dendritic cells (moDCs). On day 6, test antibodies were added for 1 hour prior to ATP addition. Post-ATP addition, cells were incubated overnight and subjected to flow cytometry the next day. (C,D) ATP dose response showing an increase in moDC maturation as evidenced by an increase in CD83 and CD86 with increasing amounts of ATP. The effect is amplified by the addition of anti-CD39 antibody. Shown with 4 donors. **B, E, F. Increased inflammasome activation by AB598 treatment in the presence of ATP.** CD14 positively selected cells from peripheral blood were differentiated to generate M0 macrophages. Concurrent CD39 inhibition with AB598 and 1 ng/mL LPS stimulation for 3 hours was followed by the addition of ATP for 4 hours. This led to an increase in IL-1β and IL-18 secretion, indicative of inflammasome activation. Data are shown from 3 donors and the error bar is the SEM. Statistics were calculated using a ratio paired t-test relative to the ATP-matched isotype control condition where *, $p < 0.05$ and ***, $p < 0.001$. IL-1β was measured by ELISA and IL-18 by MSD.

### AB598.mIgG2a Treatment Inhibits Intratumoral CD39 Enzymatic Activity *In Vivo*

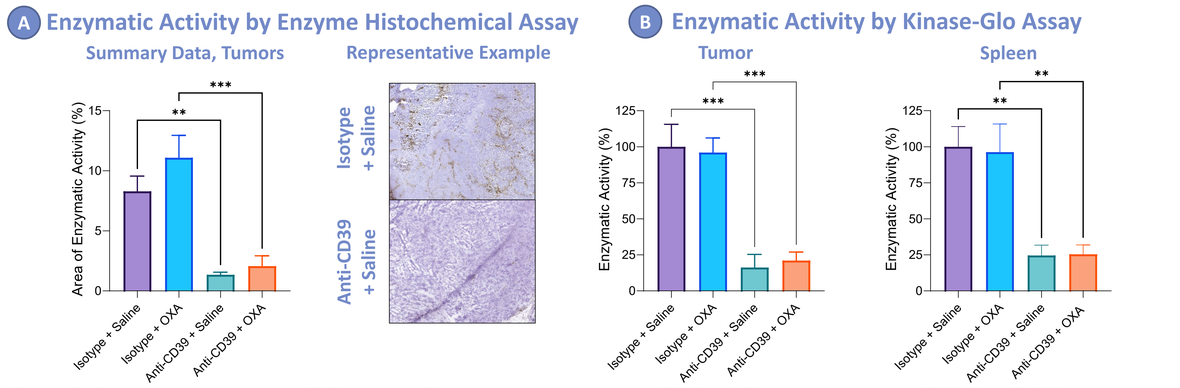
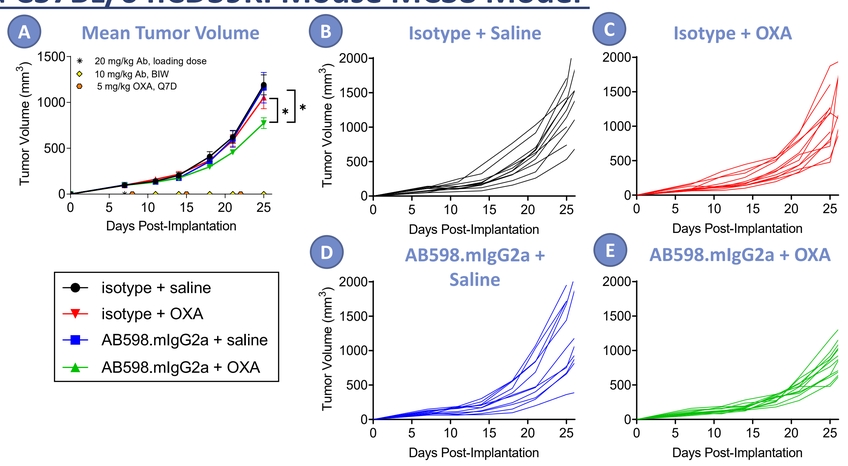



**Figure 5. Enzymatic inhibition of CD39 *in vivo*.** Tumors and spleens from AB598.mIgG2a-treated human CD39 KI (hCD39KI) mice were harvested at the conclusion of the experiment depicted below in Figure 6. **A) AB598.mIgG2a inhibits intratumoral CD39 enzymatic activity, measured by EHC.** An enzymatic histochemical (EHC) assay measured CD39 enzymatic activity by phosphate product formation by lead-phosphate deposition. The percent of CD39 enzymatic activity of the total area is shown on the y-axis. The EHC assay was conducted in the presence of 250 µM ATP and 2.5 mM levamisole, to block non-specific phosphatase activity. Tumors treated *in vivo* with AB598.mIgG2a had less lead-phosphate deposition observed by EHC, indicating that CD39 enzymatic activity had already been blocked *in vivo*. **B) AB598.mIgG2a inhibits intratumoral and splenic CD39 enzymatic activity, measured by Kinase-Glo.** Enzymatic activity was quantified using the Promega Kinase-Glo assay which measures substrate ATP levels. Tumors or spleens were digested to a single cell suspension and treated *ex vivo* with 100 nM AB598 or isotype control. 20 µM ATP was added and the difference in ATP consumption between the *ex vivo*-isotype control treated and *ex vivo*-AB598 treated samples was used to determine the *in vivo* blocking activity of AB598. The enzymatic activity (%) shown on the y-axis = (*ex vivo* AB598 RLU – *ex vivo* isotype RLU)/ Isotype + Saline Group Average (*ex vivo* AB598 – *ex vivo* isotype) *100. Data are shown from 6-7 animals/group and the error bar is the SEM. Statistics were calculated using a one-way ANOVA where **, $p \leq 0.01$ and ***, $p \leq 0.001$.

### AB598.mIgG2a + OXA Combination Shows Efficacy Compared to Single Agents in a C57BL/6 hCD39KI Mouse MC38 Model

**Figure 6. *In vivo* efficacy of AB598.mIgG2a.** C57BL/6 hCD39KI mice (GenOway) were inoculated with 1E6 MC38 cells. When tumors reached ~80 $mm^3$, mice were dosed with biweekly AB598.mIgG2a antibody, which always preceded weekly OXA dosing. The anti-CD39 antibody used *in vivo*, AB598.mIgG2a, has identical CDRs to AB598 and a murine Fc-silent constant region, mIgG2a.AAG. A) Mean tumor volume ± SEM of 11-12 animals/group and B-E) tumor volumes of individual animals. Statistical significance was calculated using a mixed-effects model with multiple comparisons, * $p \leq 0.05$.




### Solid Tumors Express the Machinery for Responding to Elevated ATP as a Result of CD39 Inhibition

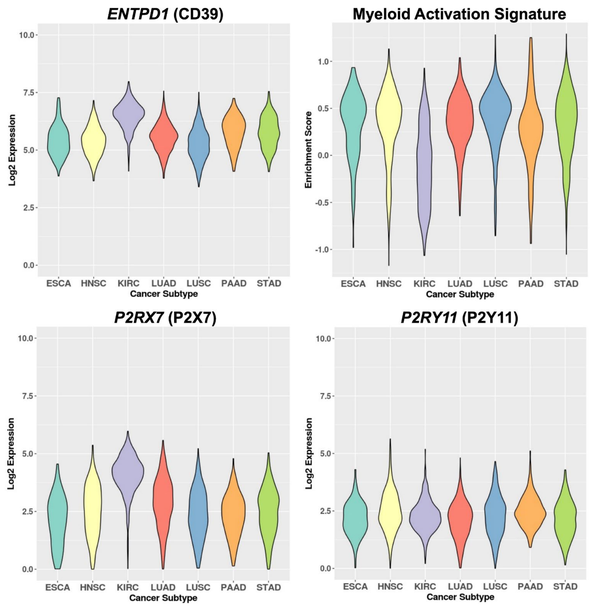



**Figure 7. ATP response machinery in solid tumors of interest.** Violin plots outline the kernel probability density (the width of the shaded area represents the proportion of the data located there) of the normalized $\log_2$ gene expression (y-axis) of the following genes (proteins): *ENTPD1* (CD39), *P2RX7* (P2X7), and *P2RY11* (P2Y11) among a subset of TCGA samples. Esophageal carcinoma (ESCA), head and neck squamous cell carcinoma (HNSC), kidney renal clear cell carcinoma (KIRC), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), pancreatic adenocarcinoma (PAAD), and stomach adenocarcinoma (STAD). Also shown is the pathway enrichment score (y-axis) for the myeloid activation signature (calculated using gene set enrichment analysis)[1] for the same subset of TCGA cancer subtypes. Using this metric, values > 0 indicate upregulation and values < 0 indicate downregulation. All tumors have high levels of CD39 and myeloid infiltration. All tumors also share similar levels of P2X7 and P2Y11, indicating they can respond to elevated ATP in the TME. [1]McDermott (2018) Nat. Med., doi:10.1038/s41591-018-0053-3.

## CONCLUSIONS

- AB598 binds primary human monocytes and B cells with sub-nanomolar affinity independently of ATP concentration.
- AB598 increases the immunogenic effect of ATP on monocyte-derived dendritic cells and macrophages to increase costimulatory molecules and pro-inflammatory cytokine production.
- *In vitro*, AB598 increases the extracellular concentration of ATP in AB598-treated tumor cells upon treatment with immunogenic chemotherapy.
- *In vivo,* murinized AB598 inhibits intratumoral CD39 and the combination of murinized AB598 + oxaliplatin reduces tumor growth.
- Analysis of RNA expression from the TCGA suggests that ATP-induced immunostimulation has the potential for broad application in solid tumors.
- Taken together, these data shows that inhibition of CD39 with AB598 in combination with an immunogenic cell death inducing agent is a potential therapeutic for the treatment of several solid tumor types.

## CONTACT

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