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Abstract 321

Inhibition of CD39 Results in Elevated ATP and Activation of Myeloid Cells to Promote Anti-Tumor Immunity

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INTRODUCTION

- CD39 (*ENTPD1*) is an ecto-nucleoside triphosphate diphosphohydrolase expressed widely in the tumor microenvironment (TME) responsible for catalyzing the conversion of ATP to AMP
- Inhibiting CD39 enzymatic activity can promote anti-tumor immune responses by increasing levels of the immunostimulatory substrate ATP
- CD39 inhibition has been shown to have an anti-tumor effect by activating dendritic cells, macrophages, and NK cells in the TME to promote antigen presentation and pro-inflammatory cytokine release
- Here we describe the preclinical characterization of AB598, a humanized Fc-silent (FcS) IgG1 anti-human CD39 antibody undergoing IND-enabling studies
- AB598 potently binds to CD39 expressed on human primary myeloid cells and tumor cells and potently inhibits CD39 enzymatic activity, leading to activation of dendritic cells and anti-tumor activity

Therapeutic Hypothesis: CD39 inhibition increases immunostimulatory ATP resulting in myeloid cell activation and anti-tumor immunity

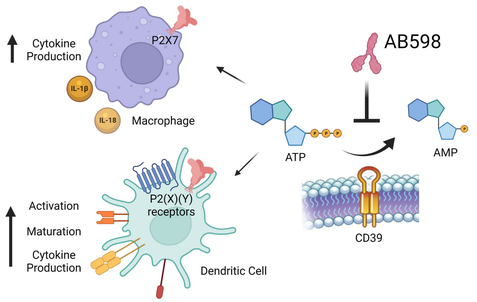


Figure 1. CD39 inhibition promotes anti-tumor immunity by myeloid cell activation. Schematic created with BioRender.com.

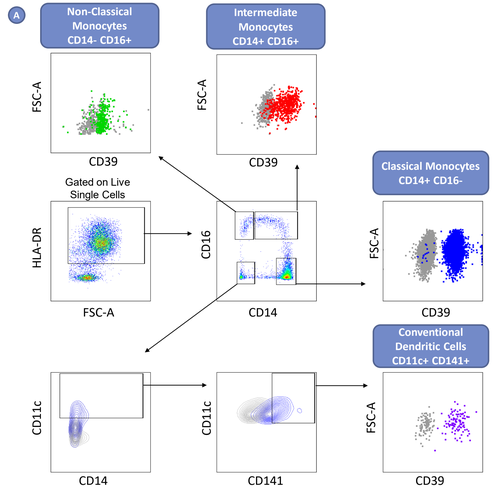
RESULTS

AB598 Potently Binds Recombinant Human CD39

	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (M)
AB598	3.0(±0.3)x10^{5}	7.2(±0.4)x10^{-5}	2.4(±0.3)x10^{-10}

Table 1. Full kinetic binding of AB598 to recombinant human CD39 by surface plasmon resonance (SPR). Measurements were collected in monovalent mode and are averaged data from seven different antibody surface densities.

CD39 is Highly Expressed on Myeloid Cells



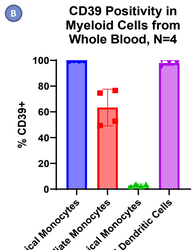


Figure 2. Expression of CD39 in myeloid cells from human subjects. (A) Representative flow plots from a single donor (whole blood). The live single cell gate also excluded cells staining positive for CD19, CD20, and CD56. CD11c, CD141, and CD39 positive populations were identified using isotype controls shown in gray. (B) Summary data from four donors showing a high percentage of CD39 positive cells across all donors in classical monocytes, intermediate monocytes, and conventional dendritic cells.

AB598 Potently Binds Cell Surface CD39

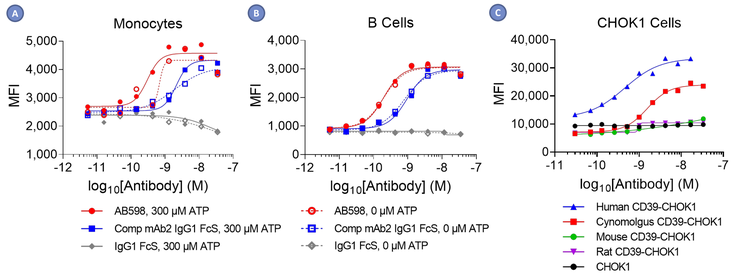


Figure 3. Binding of AB598 to cell surface CD39. Monocytes (A) were gated on CD14 positivity, and B cells (B) on CD19 positivity, to determine binding of AB598 to specific cell types in primary human PBMCs. Data representative of N=6 donors. The binding of AB598 is not affected by the presence or absence of ATP and AB598 binds with greater affinity than competitor antibody Comp mAb2 IgG1 FcS*. (C) Binding of Arcus anti-CD39 IgG4** on CHOK1 cells overexpressing human and cynomolgus CD39. No binding is observed on CHOK1 cells overexpressing mouse or rat CD39.

*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.

AB598 is a Potent Inhibitor of CD39 Enzymatic Activity

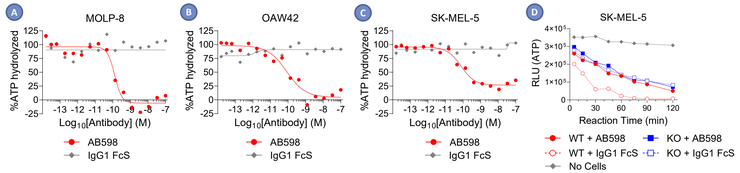


Figure 4. AB598 inhibits CD39 enzymatic activity. (A-C) AB598 inhibits enzymatic activity on human CD39-expressing cancer cell lines. Assays were conducted with 20 µM ATP and Promega Kinase-Glo™ as illustrated in Figure 6. (D) Wildtype (WT) or CD39 knockout (KO) SK-MEL-5 cells were treated with AB598 or corresponding isotype control and 20 µM ATP. SK-MEL-5 WT ATPase inhibition by AB598 resulted in an identical reduction in ATP consumption as compared to CD39 KO, indicating that AB598 fully inhibits CD39 enzymatic activity.

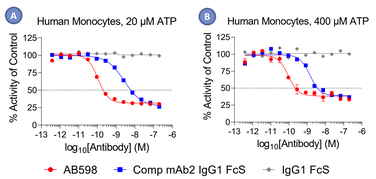


Figure 5. AB598 inhibits CD39 enzymatic activity on primary cells in low and high ATP conditions. AB598 inhibits CD39 on primary human monocytes in the presence of low, 20 µM (A) and high, 400 µM (B) ATP with a 10-20 fold increase in potency compared to a competitor antibody. Both A and B were conducted using AMP-Glo™ as illustrated in Figure 6. Representative data from N=2 donors.

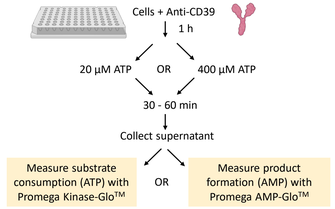


Figure 6. Enzymatic Assay Scheme. Assays carried out at 37 °C. Typically, assays with low amounts of ATP (20 µM) were conducted using Promega Kinase-Glo™ and assays with high amounts of ATP (400 µM) were conducted using Promega AMP-Glo™.

AB598 Promotes ATP-Dependent Dendritic Cell Maturation in a Monocyte-Derived Dendritic Cell System

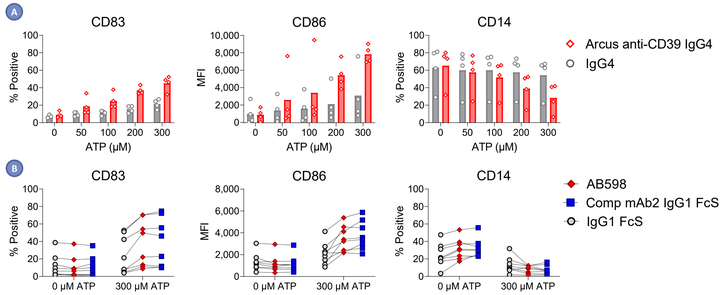
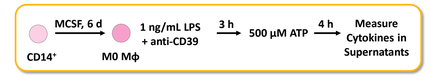


Figure 7. Increased moDC maturation by AB598 treatment in the presence of ATP. CD14 positively selected cells from peripheral blood were differentiated in GM-CSF and IL-4 for 6 days to generate monocyte-derived dendritic cells (moDCs). On day 6, test antibodies were added for 1 hour prior to ATP addition. Post-ATP addition, cells were incubated overnight and subjected to flow cytometry the next day. Top row (A): 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 four donors. Bottom row (B): Across eight donors, an increase in moDC maturation is seen with AB598 at 300 µM ATP.

AB598 Promotes Inflammasome Activation



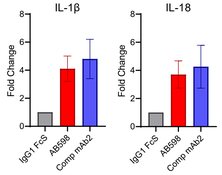


Figure 8. Increased cytokine secretion by AB598 treatment in macrophages. Concurrent CD39 inhibition with AB598 and LPS stimulation followed by ATP addition led to an increase in IL-1β and IL-18 production, indicative of inflammasome activation. Data is shown from six donors and the error bar is the SEM. Fold change was performed to normalize donor-to-donor variability and is the increase in cytokine secretion of anti-CD39 treatment compared to isotype control treatment. Cytokines were measured using ELISA.

Arcus anti-CD39 Antibody Effectively Induces Tumor Growth Inhibition in a MOLP-8 Xenograft Model

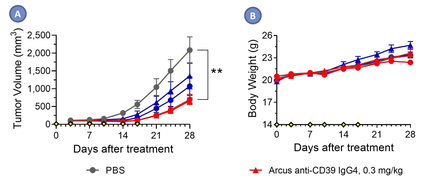


Figure 9. Anti-CD39 Single Agent Activity in MOLP-8 Xenograft Tumor Model. Two million MOLP-8 cells were injected subcutaneously on Day 0 into CB-17 SCID mice, 8 mice per group. Anti-CD39 antibody was delivered I.P. BIW for 6 doses total. A) Tumor volume and B) body weight were monitored for 28 days. Arcus anti-CD39 provided significant (**, $p < 0.01$, two-way ANOVA) tumor control at both the 0.3 and 1 mg/kg doses relative to vehicle control (PBS). Body weight increased over the course of the study indicating Arcus anti-CD39 was well tolerated.

CONCLUSIONS

- Arcus Biosciences has generated a humanized Fc-silent IgG1 anti-CD39 antibody, AB598, capable of potently binding and inhibiting enzymatic activity of human CD39 on primary human cells and cancer cells lines
- AB598 can activate myeloid cells to promote an anti-tumor phenotype, promoting maturation of moDCs and inflammasome activation in *in vitro*-derived macrophages
- *In vivo*, AB598 controls tumor growth in a MOLP-8 xenograft model

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