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Characterization of AB598, a Therapeutic Anti-Human CD39 Antibody for the Treatment of Cancer

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INTRODUCTION

- CD39 (*ENTPD1*) is an ecto-nucleoside triphosphate diphosphohydrolase expressed widely in the tumor microenvironment (TME) that catalyzes the conversion of ATP to AMP
- Inhibiting CD39 enzymatic activity can promote anti-tumor immune responses by increasing levels of the immunostimulatory substrate ATP
- AB598, a humanized Fc-silent (FcS) IgG1 anti-human CD39 antibody, 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
- Here we present pharmacodynamic characterization of AB598 *in vitro* utilizing blood-derived samples from cynomolgus monkeys, healthy human donors, and cancer patients as well as *in vivo* in cynomolgus monkeys and a human CD39 knock-in (hCD39KI) murine tumor model

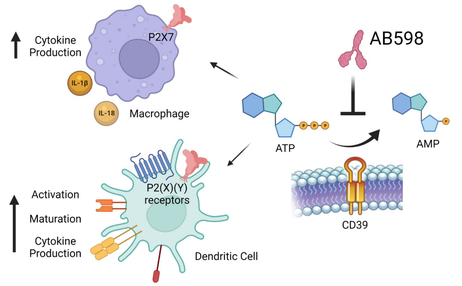

Figure 1. CD39 inhibition promotes tumor immunity. CD39 suppresses the immune system by scavenging immunostimulatory extracellular ATP released in the TME by dying cells. Inhibiting CD39 can increase levels of extracellular ATP within solid tumors resulting in myeloid cell activation and release of pro-inflammatory cytokines.

RESULTS

CD39 is Expressed by Monocytes, Granulocytes, B cells, and Other Lymphocytes in the Periphery

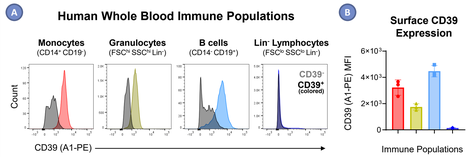

Figure 2. CD39 expression in human whole blood. (A) CD39 expression was measured on the surface of peripheral blood monocytes, granulocytes, B cells, and other lineage negative (Lin-) lymphocytes in healthy donors (n = 3) by flow cytometry. (B) Quantification of cell surface CD39 protein expression levels are shown by CD39 (A1-PE) median fluorescence intensity (MFI). Plots show group mean ± SD.

AB598 Potently Binds Cell Surface CD39 on Whole Blood Immune Populations

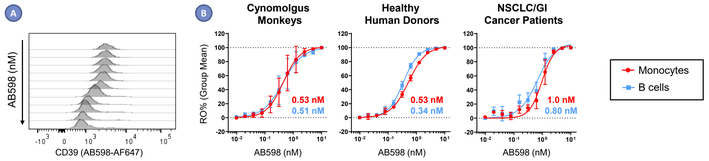

Figure 3. Potency of AB598 in whole blood from cynomolgus monkeys, healthy human donors, and cancer patients. Binding of AB598 to CD39+ immune cells was assessed by flow cytometry *in vitro* using fluorescently-labeled AB598-AF647 in a competition binding assay. CD39+ cells were identified using a non-competitive CD39 antibody (clone A1). (A) Representative histograms from a single donor show reduced binding of AB598-AF647 in the presence of increasing AB598 spike-in concentrations on CD39+ monocytes. (B) Receptor occupancy (RO) in blood from cynomolgus monkeys (n = 3), healthy human donors (n = 3), and cancer patients (n = 2) was plotted to determine EC_{50} (group mean shown). Binding affinity was similar across all CD39-expressing populations analyzed (granulocytes and Lin- lymphocytes data not shown). Plots show group mean ± SD.

Cellular and Soluble CD39 Activity and Soluble Protein Detected in Blood Samples of Healthy Individuals and Advanced Cancer Patients

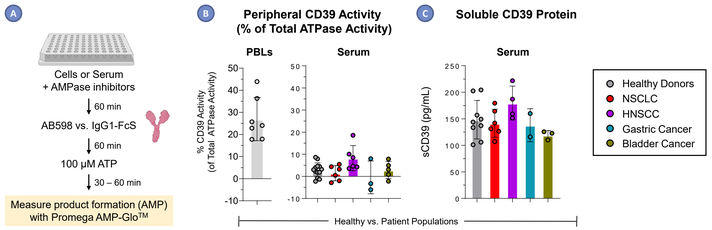

Figure 4. CD39 activity and soluble protein in peripheral blood-derived samples. (A) CD39 activity was determined using the Promega AMP-Glo assay by quantifying differences in AMP generation between AB598-treated (100 nM) and isotype-treated (100 nM) cells or serum samples. CD39 activity (ATPase activity inhibited by AB598) was calculated as a percentage of the total ATPase activity (ATPase activity inhibited by isotype). (B) % CD39 activity (of total ATPase activity) was measured in peripheral blood leukocytes (PBLs) from healthy donors (n = 7) and in serum from healthy donors (n = 13) and advanced cancer patients (n = 20). CD39 activity in PBLs is approximately 9-fold higher than CD39 activity detected in serum from healthy donors, indicating that most CD39 activity in peripheral blood is cell-associated. Overall, healthy individuals and cancer patients have similar levels of serum CD39 activity, with many individuals exhibiting very low or undetectable serum CD39 activity. (C) Soluble CD39 protein was detected at similar levels in serum from healthy individuals (n = 9) compared to advanced cancer patients across several clinical indications including NSCLC, HNSCC, gastric cancer, and bladder cancer (n = 15) by ELISA. Plots show group mean ± SD.

Surface CD39 Decreases After *In Vitro* Exposure to AB598 in Whole Blood

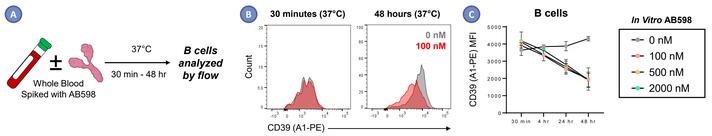

Figure 5. *In vitro* AB598 exposure in healthy donor whole blood. (A) AB598 was spiked into whole blood from healthy human donors (n = 2) at concentrations varying from 0 nM to 2000 nM. Whole blood samples were maintained in a 37°C incubator for between 30 minutes and 48 hours prior to analysis by flow cytometry to quantify surface CD39 protein levels on B cells. (B) Progressive loss of surface CD39 was observed on B cells with approximately 50% reduction in MFI observed by 48 hours. (C) Exposures to AB598 concentrations higher than 100 nM did not result in greater loss of surface CD39 on B cells. No change in surface CD39 expression was observed on B cells maintained at room temperature for up to 48 hours (data not shown). Plots show mean ± SD.

Complete Peripheral Receptor Occupancy in AB598-Dosed Cynomolgus Monkeys Associated with Decreased CD39 on Circulating Immune Cells

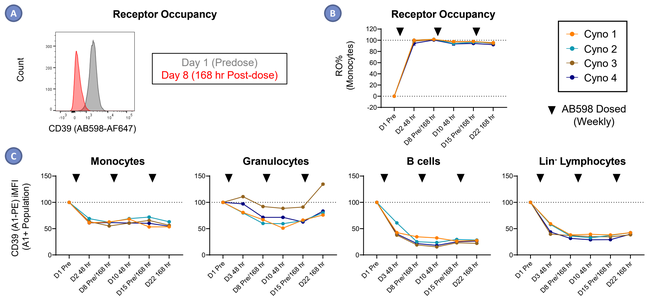

Figure 6. Peripheral target engagement and loss of surface CD39 in AB598-dosed monkeys. Naive cynomolgus monkeys (n = 2/sex) received weekly doses of AB598 i.v. for a period of three weeks. Whole blood samples were analyzed by flow cytometry for receptor occupancy (RO) by AB598 and changes in surface CD39. (A) Representative histograms from samples drawn at baseline (D1 Predose) and 168 hours following the first dose of AB598 are shown. RO was measured using fluorescently-labeled AB598-AF647 in a competition binding assay. (B) Complete RO was maintained in all monkeys across CD39-expressing immune populations (monocyte data shown) after the initial dose of AB598 until the last study timepoint. (C) Loss of surface CD39 was observed on all CD39-expressing cell populations to varying degrees, with greatest overall changes observed on B cells. Surface CD39 decreases were sustained until the end of the study.

Complete Receptor Occupancy and CD39 Enzymatic Inhibition Achieved Within Tissues of AB598-Dosed Cynomolgus Monkeys

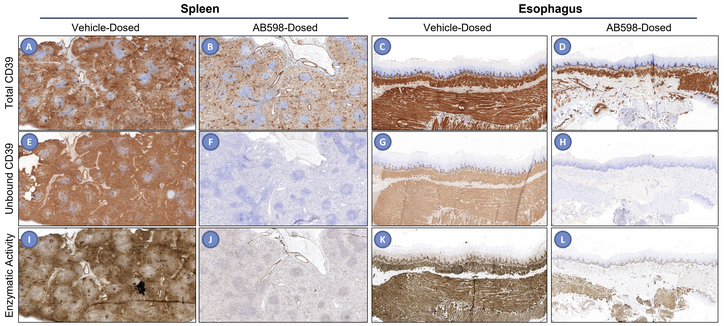

Figure 7. CD39 immunohistochemistry (IHC) and enzyme histochemistry (EHC) on spleens and esophagi from AB598-dosed monkeys. Cynomolgus monkeys received weekly i.v. doses of AB598 or vehicle for four weeks and tissues were taken at necropsy 24 hours after the last dose. (A – D) Total CD39 protein was detected by IHC using an antibody that is non-competitive with AB598 and showed CD39 expression in the spleens of both (A) vehicle and (B) AB598-dosed animals, localized to the red pulp and to a lesser degree in the white pulp. Decreased CD39 IHC intensity was observed in AB598-dosed spleens, consistent with Figure 6C. (C, D) IHC on esophagi showed CD39 staining in smooth muscle of the muscularis mucosa and externa, immune cells and vasculature in the submucosa and lamina propria, and mucosal intraepithelial immune cells. (E – H) IHC for unbound CD39 was performed using AB598.mIgG2a, which competes for the same epitope and showed a staining pattern consistent with the total CD39 IHC in (E, G) vehicle-dosed tissues and no staining in (F, H) AB598-dosed tissues, indicating full target engagement in the AB598-dosed tissues. (I – L) CD39 enzymatic activity was evaluated by lead-phosphate deposition, conducted in the presence of substrate and a non-specific phosphatase inhibitor. Tissues from (I, K) vehicle-dosed animals showed high levels of lead-phosphate deposition, matching the expression pattern of CD39, while (J, L) AB598-dosed animals showed lead-phosphate signal reduced to non-CD39 dependent levels, indicating full target inhibition.

Complete Peripheral RO, Intratumoral Enzymatic Inhibition, and Decreased Cell Surface Human CD39 with Anti-CD39 Administration

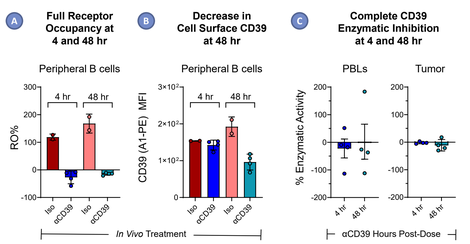

Figure 8. Pharmacodynamic assessment of AB598.mIgG2a in mice. C57BL/6 human CD39 knock-in (hCD39KI) mice (Biocytogen) were implanted with MC38 tumors. When tumors reached 175 – 600 mm^3, mice were dosed i.v. with 10 mg/kg AB598.mIgG2a, an anti-CD39 antibody that has identical CDRs to AB598 and a mutated murine IgG2a Fc-silent constant region, or isotype control antibody. (A) Receptor occupancy (RO) and (B) surface CD39 were measured on peripheral immune cells (B cell data shown). Complete RO was achieved by 4 hours while changes in surface CD39 appeared by 48 hours. (C) Complete enzymatic inhibition was detected in peripheral blood leukocytes (PBLs) and dissociated tumor cells using Kinase-Glo (Promega) at 4 and 48 hours after anti-CD39 treatment. Plots show group mean ± SD. Points represent individual animals.

CONCLUSIONS

- AB598, a humanized Fc-silent IgG1 anti-CD39 antibody, potently binds CD39 on whole blood immune cells from cynomolgus monkeys, healthy human donors, and cancer patients
- Cellular CD39 activity constitutes the majority of peripheral CD39 activity; soluble CD39 activity and protein were detected at similar levels in serum from healthy donor versus cancer patients
- Human B cells exposed to AB598 *in vitro* in whole blood and maintained at physiological conditions for 48 hours progressively lose surface CD39
- AB598-dosed monkeys maintained complete receptor occupancy in the periphery and within tissues, as well as complete inhibition of CD39 activity within tissue samples; loss of surface CD39 was also observed on all CD39-expressing peripheral immune cell types and immune cell-rich tissues
- In hCD39KI mice bearing MC38 tumors, complete peripheral receptor occupancy and both peripheral and intratumoral inhibition of CD39 enzymatic activity are observed within 4 hours, while loss of surface hCD39 on peripheral immune cells is observed by 48 hours post-dose
- ***Taken together, these data highlight the distribution of CD39 in peripheral blood and tissues and demonstrate consistent pharmacodynamic effects of AB598 in preclinical settings, including its ability to fully engage CD39, inhibit activity, and trigger loss of CD39 from the surface of immune cells.***

CONTACT

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