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

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ABSTRACT

AB598, a humanized Fc-tail IgG1 anti-human CD39 antibody, potently binds to CD39 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 human CD39 knock-in (hCD39KI) murine tumor models.

INTRODUCTION

CD39 (ENTPD1) is an extracellular triphosphate dephosphohydrolase expressed ubiquitously 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-tail IgG1 anti-human CD39 antibody, potently binds to CD39 on human primary myeloid cells and tumor cells and potently inhibits CD39 enzymatic activity, leading to activation of dendritic cells and anti-tumor activity.

RESULTS

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

Human White Blood Immune Populations

Surface CD39 Expression

Table 1: CD39 Expression on Human White Blood Immune Cells

AB598 Potently Binds Cell Surface CD39 on Whole Blood Immune Populations

Figure 1: CD39 inhibition promotes tumor immunity. CD39 suppresses the immune system resulting in inhibition of anti-tumor immune responses by increasing levels of the immunostimulatory substrate ATP.

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

Figure 2: CD39 activity and cellular protein in peripheral blood-derived samples. (A) CD39 activity was determined using the Promega AMP-Glo assay for quantifying differences in ATFase generation between AB598-treated (100 nM) and vehicle-treated cells in vitro. CD39 enzymatic activity (ATPase activity inhibited by AB598) was calculated as a percentage of the total ATPase activity (ATPase activity inhibited by isotype). (B) % of healthy donors, indicating that most CD39 activity in peripheral blood is cell-associated. Overall, healthy individuals and cancer patients have 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 24 hours, with approximately 50% reduction in MFI observed by 48 hours.

Complete Peri pheral Receptor Occupancy in AB598-Dosed Cynomolgus Monkeys Associated with Decreased CD95/CD39 Circulating Immune Cells

Figure 3: Peripheral CD39 activity and loss of surface CD95 in AB598-dosed monkeys. Macrophage-monocyte (M) and neutrophil (N) peripheral immune cells from cynomolgus monkeys, healthy human donors, and cancer patients were analyzed by flow cytometry for receptor occupancy (RO) by AB598 and percentage of cells with reduced CD5 and CD39 expression. In hCD39KI mice bearing MC38 tumors, complete peripheral receptor occupancy and both peripheral and intratumoral inhibition of CD39 enzymatic activity was observed within 4 hours, while loss of surface CD95 on peripheral immune cells is observed by 48 hours post-dose.

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

Figure 4: Pharmacodynamic response of AB598恩 in mice. (A) Mice bearing human CD39 knock-in (hCD39KI) tumors were treated i.p. with 30 μg/kg AB598-af647, 30 min before excision at 120 min. Whole tumor tissues were analyzed by flow cytometry for receptor occupancy (RO) by AB598 and percentage of cells with reduced CD5 and CD39 expression. (B) Macrophage-monocyte (M) and neutrophil (N) peripheral immune cells from cynomolgus monkeys, healthy human donors, and cancer patients were analyzed by flow cytometry for receptor occupancy (RO) by AB598 and percentage of cells with reduced CD5 and CD39 expression.

CONCLUSIONS

AB598, a humanized Fc-tail IgG1 anti-CD39 antibody, potently binds to 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, while soluble CD39 protein and percent 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, with significant decreases observed within 4 hours.

AB598-dosed monkeys maintained complete receptor occupancy in circulating immune cells present in peripheral blood and within tissues, as well as complete inhibition of CD95 activity within tissue samples, loss of surface CD95 was also observed on CD95-expressing peripheral immune cell types and immune cell subtypes.

In hCD39KI mice bearing MC38 tumors, complete peripheral receptor occupancy and both peripheral and intratumoral inhibition of CD39 enzymatic activity was observed within 4 hours, while loss of surface FcαRI on peripheral immune cells is observed by 48 hours post-dose.

Tumor response to AB598 administration is likely driven by AB598 peripheral and tissues and demonstrates consistent pharmacodynamic effects of AB598 in preclinical settings, including its ability to fully engage CD95, inhibit activity, and trigger loss of CD95 from the surface of immune cells.