**AB474, A Potent Orally Bioavailable Inhibitor of Arginase, for the Treatment of Cancer**

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**Introduction**

It has been demonstrated that myeloid derived suppressor cells (MDSCs) have a direct role in tumor immune evasion, and increased MDSCs are associated with reduced overall survival in several types of cancer. Elevated levels of circulating MDSCs have been shown to correlate with a blunted response to checkpoint blockade. MDSCs secrete arginase, which depletes arginine, leading to decreased T-cell activity and a suppressed anti-tumor response. AB474 inhibits arginase with low-nanomolar potency. It effectively inhibits both recombiant and endogenous arginase, while displaying low protein binding across species. Pharmacokinetic characterization of AB474 demonstrated oral bioavailability in mice, rats, dogs and monkeys, consistent with expected good oral bioavailability in humans. Measuring transcript of Arg1 in murine tumor models revealed expression in both tumor and immune compartments. Flow cytometry analysis shows Arg1+ MDSCs exist in both the tumor and the periphery, with higher expression levels seen in Ly6C− mMDSC populations. Consistent with the elevation of arginase in tumor-bearing mice, levels of arginase are decreased when compared to naive littersmates. Addition of arginase to activated CD4 or CD8 T cells results in a significant suppression of proliferation, CD3 expression, and IFNγ secretion. Under these conditions, AB474 potently restores normal T-cell effector functions (P < 0.001).

**Materials and Methods**

Arginase inhibition: Arginase activity was measured using a coupled enzyme assay in buffer or human serum albumin/α-T casein glycoprotein (HSA/AAG). IC50 values were determined in human plasma and serum by LC-MS/MS quantification of ornithine, while plasma protein binding was determined by equilibrium dialysis.

Mouse tumor analysis: Mouse syngeneic tumors were removed and homogenized for flow cytometry using the gentleMACS dissociator. RNA was extracted from whole mouse tumors lysed with an Omni Bead Ruptor and analyzed using Taqman probes

T cell activation assay: Arginase inhibition by AB474 was tested using isolated human CD4 and CD8 T cells from healthy donors. Proliferation was measured by flow cytometry and IFNγ secretion by ELISA. Cells were activated using CD3/CD28 beads.

Whole blood: CD4 T cells and pan-granulocytes were isolated from healthy donors or cancer patients and Arginase expression was analyzed by flow cytometry. Circulating Arginase levels were determined using a human Arginase 1 (ARG1) ELISA from BioVendor.

**Results**

**AB474 Inhibits Endogenous and Recombinant Arginase Equally Well**

A) Dose response curve of AB474 inhibition of human granulocyte arginase. Endogenous arginase activity was determined by comparing steady state initial velocity with recombinant human Arg1 protein. IC50 values were determined as 9.4 ± 0.6 mM, 9.9 ± 0.9 mM, and 10.8 ± 0.5 mM in each of three donors.

**PMN MDSCs Express Highest ARG1 Levels in Human Whole Blood**

A) ARG1 expression was determined in whole blood MDSC subsets using flow cytometry. B) PMN-MDSCs are found in both healthy donors (H1, H2) and cancer patients (P1). ARG1 MFI is consistent between healthy donors and cancer patients among the MDSC subsets. C) Circulating ARG1 levels were determined by ELISA; cancer patients show higher ARG1 levels compared to healthy donors.

**Conclusions**

- AB474 is a potent orally bioavailable inhibitor of arginase.
- Arginase depletes arginine from the local environment and suppresses anti-tumor responses.
- AB474 reverses arginase-mediated suppression of CD8 T-cell proliferation and cytokine production.
- Inhibition of arginase blocks one of the primary immunosuppressive functions of MDSCs, resulting in restored T-cell activation.

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