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Abstract # 3862

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

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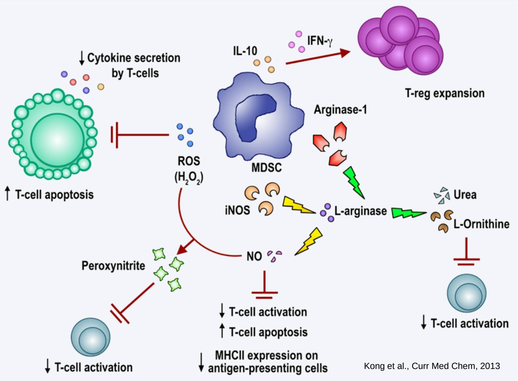
Introduction

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 recombinant and endogenous arginase, while displaying low plasma 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 arginine are decreased when compared to naïve littermates. 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).



Kong et al., Curr Med Chem, 2013

Three Mechanisms of Suppression by MDSCs:

- ROS Production
- Nutrient Depletion
- Cell:Cell Contact

Materials and Methods

Arginase inhibition: Arginase activity was measured using a coupled enzyme assay in buffer or human serum albumin/alpha 1-acid glycoprotein (HSA/AAG). IC_{50} values were determined in human plasma and serum by LC-MS/MS quantification of ornithine, while plasma protein binding was determined by equilibrium dialysis.

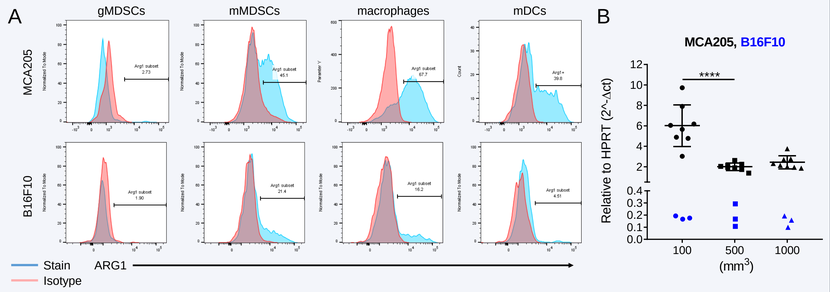
Mouse tumor analysis: Mouse syngeneic tumors were removed and homogenized for flow cytometry using the gentleMACs dissociator from Miltenyi. RNA was extracted from whole murine tumors lysed with an Omni Bead Ruptor and analyzed using Taqman probes

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 CD2/CD3/28 beads.

Whole blood: CD8 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.

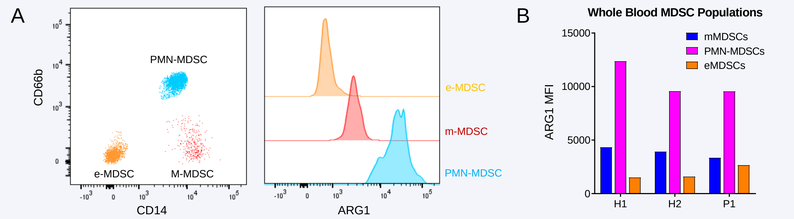
Results

ARG1 is Expressed by Multiple Immune Cell Types in Mouse Tumors



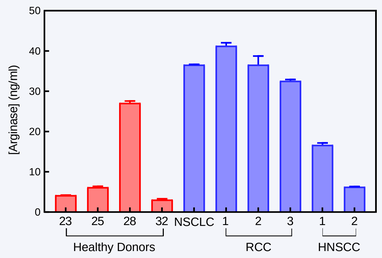
A) ARG1 staining by flow cytometry on homogenized MCA205 and B16F10 tumor models shows staining mMDSCs, macrophages, and mDCs. B) Highest ARG1 transcript levels are observed in 100 mm^3 tumors from the MCA205 model.

PMN MDSCs Express Highest ARG1 Levels in Human Whole Blood



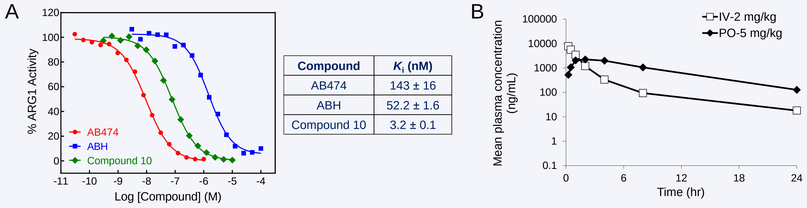
C

	[Arginase] (ng/ml)	[Arginase] (nM)
D23	4.2	0.1
D25	6.2	0.17
D28	27.1	0.75
D32	3.1	0.1
NSCLC	36.6	1.0
RCC1	41.3	1.2
RCC2	36.6	1.0
RCC3	32.6	0.9
HNSCC1	16.7	0.5
HNSCC2	6.3	0.2



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.

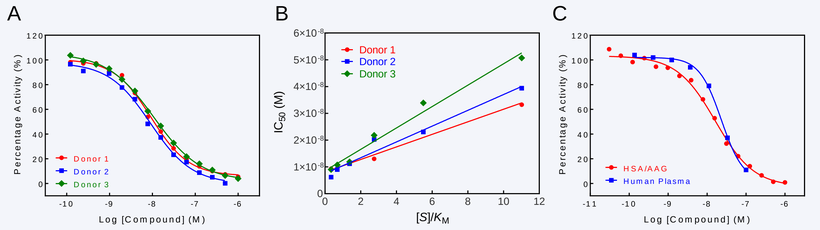
AB474 Inhibits Human ARG1 Activity and is Orally Bioavailable



Compound	K_i (nM)
AB474	143 ± 16
ABH	52.2 ± 1.6
Compound 10	3.2 ± 0.1

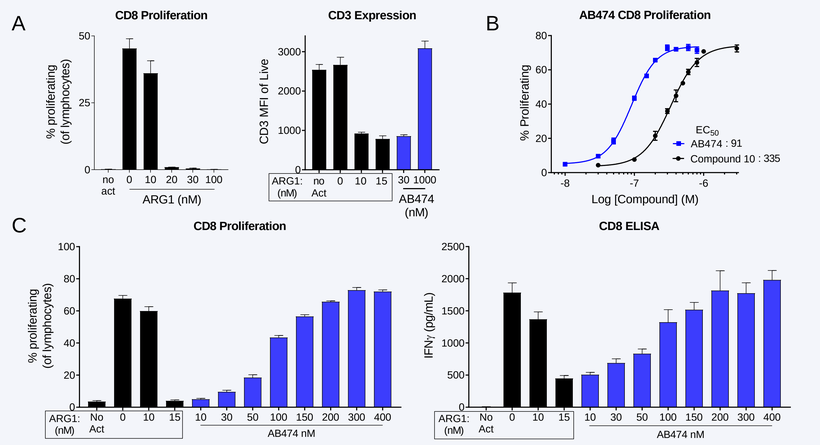
A) Dose response curve of ARG1 inhibitors on recombinant ARG1 activity. Table shows K_i values as determined by Cheng-Prusoff analysis. Compound 10 from US Pat. Appl. WO2017/075363. B) AB474 exhibits low clearance and good oral bioavailability in dogs.

AB474 Inhibits Endogenous and Recombinant ARG1 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. IC_{50} values were determined as 9.4 ± 0.6 nM, 9.0 ± 0.9 nM and 10.8 ± 0.5 nM in each of three donors. B) Cheng-Prusoff analysis of $[S]/K_m$ dependent IC_{50} for arginase prepared from human granulocyte lysate from three donors. Linear relationship suggests AB474 is a competitive inhibitor. C) Dose response curve of inhibition by AB474 of MTO activity catalyzed by human ARG1 protein in the presence of HSA/AAG or human plasma. Potency of AB474 in human plasma was measured in human plasma spiked with 0.5 nM human ARG1 protein and 0.8 mM $^{13}C_6$ L-arginine at 37°C. The values of IC_{50} are 16.5 ± 1.7 nM and 23.2 ± 2.3 nM for the potency in HSA/AAG and human plasma, respectively.

ARG1-Mediated Suppression of T-cell Activity is Inhibited by AB474



A) CD8 T cells isolated from healthy patient blood were activated using CD2/CD3/CD28 activation beads in the presence of increasing amounts of recombinant human ARG1. Levels of ARG1 over 15 nM completely suppress T-cell proliferation when measured by flow cytometry. Surface expression of CD3 decreases significantly on T cells with addition of ARG1, but levels are restored when ARG1 is inhibited. B) AB474 is highly potent and restores proliferation in T cells, with an EC_{50} of 91 nM. C) CD8 T-cell proliferation and IFNγ production are restored by AB474 in a dose dependent manner.

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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