Preclinical Pharmacokinetic and Pharmacodynamic Characterization of AB680, a Small-Molecule CD73 Inhibitor for Cancer Immunotherapy

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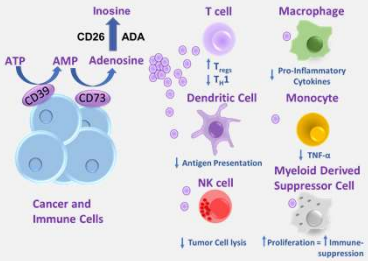
Introduction

Extracellular adenosine (ADO), present at high concentrations in the tumor micro-environment (TME), suppresses immune function via inhibition of T cell, natural killer (NK) cell, and dendritic cell (DC) activation. Intra-tumoral generation of ADO depends on the sequential catabolism of ATP by two ecto-nucleotidases: CD39 (ATP→AMP) and CD73 (AMP→ADO). Inhibition of CD73 eliminates a major pathway of ADO production in the TME and can reverse ADO-mediated immune suppression. Although less efficient, tissue non-specific alkaline phosphatases (TNAP) could also contribute to ADO generation from AMP. Here we present the preclinical characterization of AB680, a novel, highly potent, reversible and selective small molecule inhibitor of CD73, currently in preclinical development as a potential anti-tumor agent. The link between CD73 levels present in different tissues, efficacy in mouse tumor models, plasma and tumor exposure, and projected human pharmacokinetic (PK) profile can be combined to provide an expected AB680 dosing strategy for the upcoming first-in-human clinical trial.



Methods

The potency of AB680 against human CD73 was determined in CHO-CD73 cells, blood CD8⁺ T cells, recombinant CD73, and serum/plasma using either malachite green assay, AMP-Glo assay, or LC MS/MS. The selectivity of AB680 against related ecto-nucleotidases was also assessed using similar methods. Quantitation of soluble CD73 in human serum was performed via in-house developed and validated ELISA. Syngeneic mouse tumor models were established to assess the efficacy of AB680 at multiple doses. AB680 levels in plasma and tumor associated with each dosing regimen were determined via LC-MS/MS. The potency of AB680 in tumor tissues was determined using biochemical conversion of $^{13}C_5$-AMP to $^{13}C_5$-Adenosine. The effects of AB680 on syngeneic tumor volumes were assessed in prophylactic and therapeutic settings. The PK properties of AB680 were evaluated in multiple preclinical species and a projected human dosing schedule for AB680 was determined via allometric scaling.

AB680 Potency and Selectivity

Human; Buffer IC_{50} (nM)	CHO.hCD73	0.07
	rhCD73	0.0325
	CD8+ T cell	0.0084
	CD4+ T cell	0.0136
Mouse; Buffer IC_{50} (nM)	E0771	1.82
	CD8+ T cell	0.66
Plasma IC_{50} (nM)	Human	19.9
	Mouse	790

AB680 Selectivity	
Target	IC_{50} (nM)
CD39	> 10,000
A2aR	> 10,000
TNAP	> 10,000
NTPDase 2	> 10,000
NTPDase 3	> 10,000
NTPDase 8	> 10,000

Figure 1. Potency of AB680 in buffer was determine using the Malachite Green Assay. Potency of AB680 in plasma was determined using LC-MS/MS by measuring conversion of $^{13}C_5$-AMP to $^{13}C_5$-Adenosine. Selectivity of AB680 against TNAP was performed using recombinant human TNAP, while selectivity against the remaining enzymes and $A_{2a}R$ was determined using CHO transient overexpression systems.

AB680 Limits CD73 Activity and Enhances the Activity of α-PD-1 in B16F10 Melanoma Model

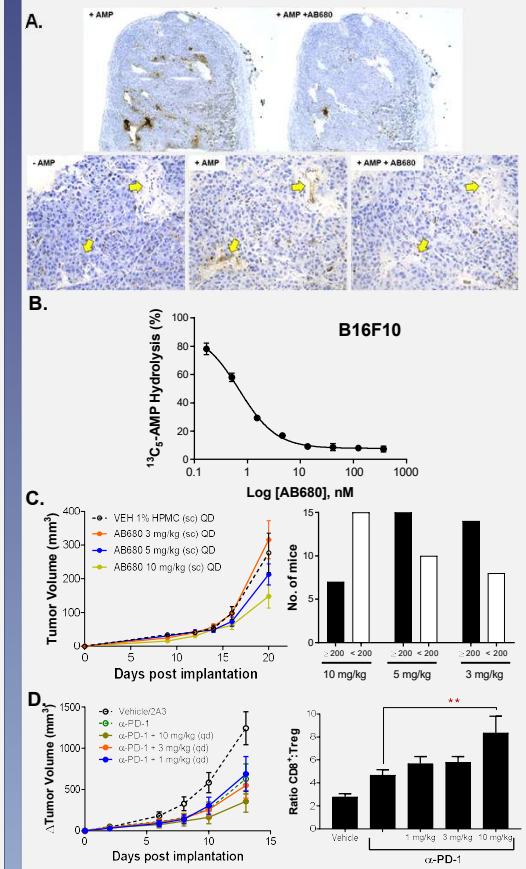


Figure 2. (A) AMPase activity was assessed on B16F10 melanoma tumor sections using a modified form of the Wachstein-Meisel method of enzyme histochemistry. Yellow arrows indicate regions with active AMP hydrolysis. **(B)** Activity of AB680 against B16F10 tumor homogenates were quantified using $^{13}C_5$-AMP hydrolysis method. Briefly, implanted tumors were excised, snap frozen, and homogenized in protein lysis buffer. AB680 was added to exogenous tumor homogenates and activity was quantified using MS. AB680 potency (IC_{50}) was less than 1 nM. **(C)** AB680 was dosed once daily on the same day of B16F10 tumor implantation. *In life* tumor measurements using calipers are shown (left graph). The number of mice with tumor volumes <200 mm³ or ≥200 mm³ at the end of the study is depicted (right bar graph). **(D)** Combined efficacy of AB680 with α-PD-1 was tested in B16F10 model using *in life* tumor measurements (left graph) and CD8 to Treg ratio in tumor infiltrating lymphocytes (right graph). Dosing was initiated when tumor volume reached ~50 mm³.

AB680 Mouse Pharmacokinetics

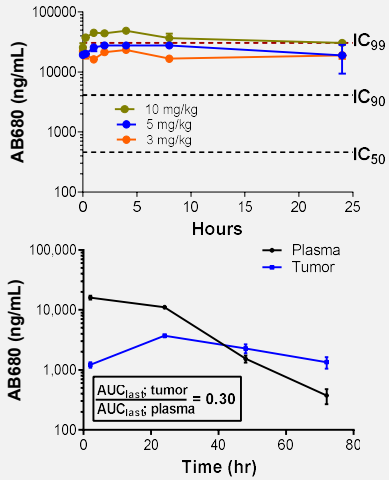


Figure 3. (A) Steady-state plasma levels of AB680 in tumor bearing mice were measured using LC-MS/MS. **Dashed lines refer to the potency of AB680 against CD73 in mouse plasma. (B)** Plasma to tumor partitioning of AB680 (10 mg/kg dose) was calculated using AUC from t = 0 to t = 72 hrs. Comparable ratios were obtained from $AUC_{0\text{-}24}$. Analysis of mouse PK/PD suggests that the effective dose of AB680 is associated with plasma levels ≥ 30,000 ng/mL. These plasma levels provide ~99% coverage in mouse plasma and an estimated 95% coverage in B16F10 tumors. Adjusting for inter-species potency differences, the corresponding human plasma concentrations would be ~ 770 ng/mL. This target exposure may represent the upper end of the range where we expect to see CD73-relatd biological effects in humans

Predicted Human Pharmacokinetics

A.

	AB680 Pre-clinical Pharmacokinetic Parameters			
	Clearance (L/h/kg)	Vss (L/kg)	Half-life (hr)	Free Fraction (%)
Mouse	0.025	0.12	3.5	0.89
Rat	0.02	0.12	5.3	0.17
Dog	0.05	1.3	21	8.3
Monkey	0.0025	0.1	27	0.59
Human	0.0012	0.17	98	0.42

B.

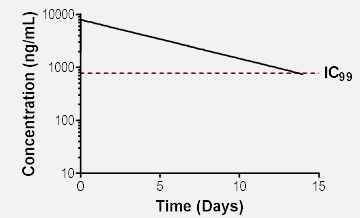


Figure 4. (A) Predicted human PK parameters were derived by allometric scaling. Vss prediction was determine by Øie-Tozer method. **(B)** Predicted human plasma profile shown was determined assuming 89 mg intravenous infusion for 1 hr resulting in 2-week trough concentration of 772 ng/mL.

Correlation Between Serum CD73 Concentration and Activity

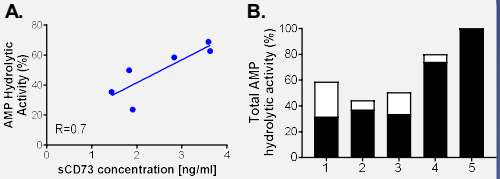


Figure 5. (A) Soluble CD73 (sCD73) levels in human serum were quantified using our in-house developed ELISA. AMP degradation was measured using AMP-Glo assay. Each dot represents one independent donor. **(B)** Total AMP hydrolysis in serum from cancer patients (1-5) were measured. Black bars represent CD73-mediated hydrolysis while white bars represent TNAP-mediated hydrolysis.

AB680 Limits the Inhibitory Effect of AMP on CD4⁺ and CD8⁺ T cells

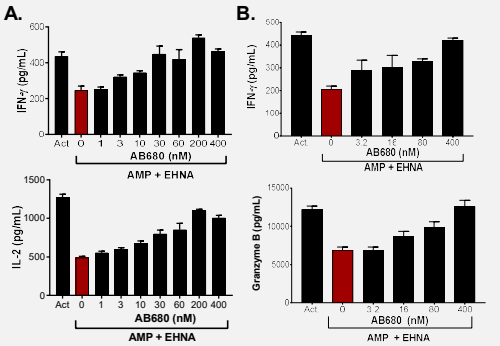


Figure 6: Human CD4⁺ T cells **(A)** and CD8⁺ T cells **(B)** were isolated and activated in the presence of AMP with EHNA (adenosine deaminase inhibitor). Dose-dependent rescue of CD4⁺ and CD8⁺ T cell activation was observed in the presence of exogenous AB680. CD4⁺ and CD8⁺ T cell activation was measured by cytokine bead array for IFN-γ and IL-2 or IFN-γ and granzyme B, respectively.

Conclusions

AB680 is a highly potent and selective small-molecule inhibitor of CD73 which can mitigate AMP and ADO-mediated tumor immunosuppression by potently blocking the production of ADO.

AB680 retains high potency when tested in human serum (potency reflects both plasma protein binding and binding to soluble CD73).

AB680 exhibits a unique projected human PK profile (including a long projected half-life) amenable to intravenous dosing schedules.

In vivo mouse studies with AB680 in B16F10 tumors suggest that >95% intra-tumoral CD73 inhibition results in increased efficacy in combination with α-PD-1; additional models to be tested.

AB680 is expected to enter clinical development in 4Q2018.