Discovery and Characterization of AB680, a Potent and Selective Small-Molecule CD73 Inhibitor for Cancer Immunotherapy

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#1756

Introduction

Extracellular adenosine (ADO) is present in high concentrations in the tumor micro-environment (TME) and suppresses immune function via inhibition of immune cell 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, non-redundant, pathway of ADO production in the TME and can reverse ADO-mediated immune suppression. Here we present the 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 *in vivo* pharmacology (i.e., effects on tumor growth and TIL landscape) are described elsewhere (see Becker et al., Abstract #3501, this meeting).

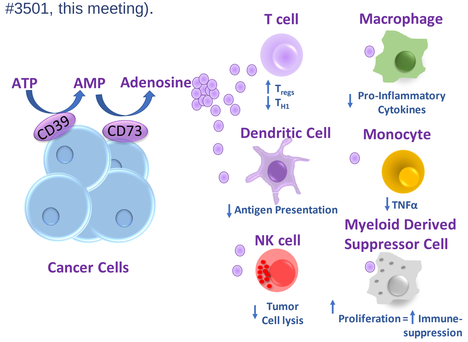


Figure 1. Adenosine, a potent immune suppressor, is generated through sequential hydrolysis of ATP by CD39 and CD73. Extracellular adenosine is removed from the microenvironment by adenosine deaminase (ADA) tethered to the cell surface by CD26.

Methods

Small molecule inhibitors of CD73 were designed by the Medicinal Chemistry Department at Arcus Biosciences leading to the discovery of AB680. Ecto-nucleotidase activity was evaluated by measuring AMP hydrolysis by CHO-CD73 cells using a malachite green assay. Potency was also measured using human T-cells and soluble recombinant CD73. The ability of AB680 to reverse AMP-mediated immune suppression of human CD4$^+$/CD8$^+$ T cells was determined by adding exogenous AMP during T cell activation by anti-CD3/CD28/CD2 beads.

Results

AB680 *in vitro* Potency

AB680 is a highly potent (K_i hCD73 = 4.9 pM), reversible and selective inhibitor of CD73. AB680 is > 10,000-fold selective against related ecto-nucleotidases (CD39, NTPDases 2/3/8) and a large panel of unrelated enzymes, receptors, and ion channels.

CD73 Potency		Selectivity	
Target	**IC_{50} (nM)**	**Target**	**IC_{50} (nM)**
hCD73-CHO	0.070	CD39	> 10,000
hCD73 (soluble)	0.043	$A_{2a}R$	> 10,000
Human CD8$^+$ T Cells	0.008	NTPDase 2	> 10,000
Mouse CD8$^+$ T Cells	0.66	NTPDase 3	> 10,000
hPBMC	0.011	NTPDase 8	> 10,000

Table 1. AB680 *in vitro* potency and selectivity summary. Selectivity assays used over expressing CHO cell lines.

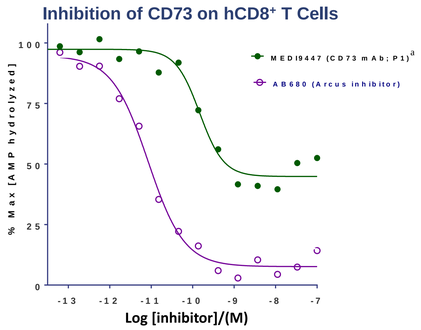


Figure 2. Dose-response of AB680 in human CD8$^+$ T cells. In contrast to anti-CD73 mAb MEDI9447, AB680 is able to completely inhibit CD73 enzymatic activity on CD8$^+$ T Cells. [a]Antibody synthesized by Arcus based on the following publication and patent application: Hay et al., OncoImmunology (2016) 5, e1208875; Patent Appl. US 2016/0129108

AB680 Restores CD4$^+$/CD8$^+$ T Cell Function

In the presence of high concentrations of AMP, AB680 robustly restores proliferation of human CD4$^+$ T-cells and reverses AMP-mediated inhibition of IFN-γ and IL-2 production. Additionally, AB680 potently restored CD25 expression on human CD8$^+$ T cells.

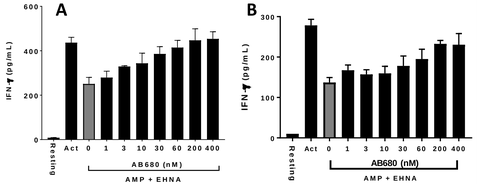


Figure 3. AB680 restores AMP-suppressed IFN-γ production in **(A)** CD4$^+$ and **(B)** CD8$^+$ T cells

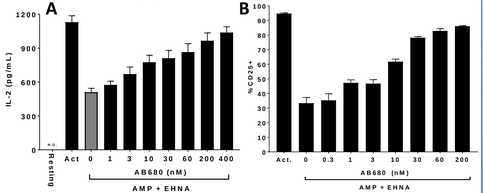


Figure 4. AB680 restores **(A)** IL-2 production on CD4$^+$ T cells and **(B)** CD25 expression on CD8$^+$ T cells.

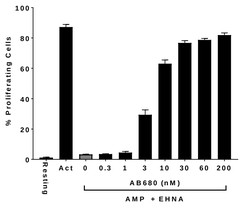


Figure 5. AB680 limits the inhibitory effect of AMP on CD4$^+$ T cell proliferation (IC_{50} = 4.5 nM).

Preclinical Pharmacokinetics

The PK properties of AB680 are characterized by very low clearance and long half-lives in preclinical species. AB680 exhibited no competitive or time-dependent inhibition of any major CYP isoforms. Furthermore, AB680 is stable in human hepatocytes and plasma.

Preclinical Pharmacokinetics *(cont.)*

Table 2. Competitive inhibition of major CYP isoforms.

CYP Isoform	1A2	2C9	2C19	2D6	3A4
IC_{50} (μM)	>100	>100	>100	>100	>100

Table 3. Summary of hepatocyte stability in various species.

Hepatocyte Stability	Mouse	Rat	Dog	Human
$T_{1/2}$ (min)	158	163	167	225
CL_{int} (μL/min/10^6 cells)	8.8	8.5	8.3	6.2

Table 4. Summary of experimental PK parameters in mouse, rat, dog, and cynomolgus monkey.

AB680 PK Parameters				
Species	**CL (L/h/kg)**	**Vss (L/kg)**	**$T_{1/2}$ (h)**	**fu (%)**
mouse	0.025	0.12	3.5	0.89
rat	0.020	0.12	5.3	0.17
dog	0.050	1.3	21	8.3
monkey	0.0025	0.10	27	0.59

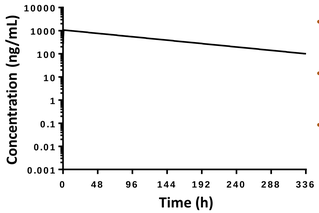
Predicted Human Pharmacokinetics

AB680 is projected to have a long human half-life (≥4 days), suitable for Q2W intravenous (i.v.) dosing. Trough concentration at two weeks are predicted to well exceed IC_{90} in serum.

Parameters	Value
CL (L/hr/kg)	0.0012
V_{ss} (L/kg)	0.17
$T_{1/2}$ (hr)	98 (4 days)

Table 5. Projected human PK parameters determined by allometric scaling. V_{ss} prediction determined by Øie-Tozer method. Most conservative prediction shown.

Predicted Human Pharmacokinetics *(cont.)*



- **Dose:** 13 mg IV infusion for 1 h
- **Peak concentration:** 1,060 ng/mL
- **Trough concentration at two weeks:** 100 ng/mL

Figure 6. Projected two-week human PK profile following an i.v. infusion of 13 mg AB680.

Preclinical Toxicology

Preliminary toxicology studies show AB680 to be well tolerated following high-dose i.v. infusions in rats. Daily observations (e.g. body weight) did not highlight any difference between dosed animals and vehicle up to 100 mg/kg.

AB680 Rat TK Summary		
Dose (mg/kg)	**C_{max} (ng/mL)**	**AUC (ng•h/mL)**
5	85,750	644,500
25	231,500	2,130,000
100	1,185,000	9,200,000

Table 6. Summary of AB680 exposure in rats following a 5, 25, or 100 mg/kg i.v. infusion (30 min). Animals were dosed Q3D (*n* = 3M/3F per dose group). AB680 exposure following dose 3 was within standard deviation of dose 1 levels.

Summary

- AB680 is a highly potent and selective small-molecule inhibitor of CD73 which can mitigate AMP and ADO-mediated inhibition of T cell activation. Similar effects in the TME are expected to translate into enhanced anti-tumor immunity.
- AB680 exhibits a favorable projected human PK profile suitable for parental administration on a schedule consistent with typical mAb dosing cycles.
- AB680 is expected to enter clinical development in 2018.