Selection of Optimized Drug Candidates, Dosing Regimen, Pharmacodynamic Endpoints, Tumor Types, and Biomarkers for Translating Inhibition of the Adenosine Pathway into Effective Anti-tumor Activity

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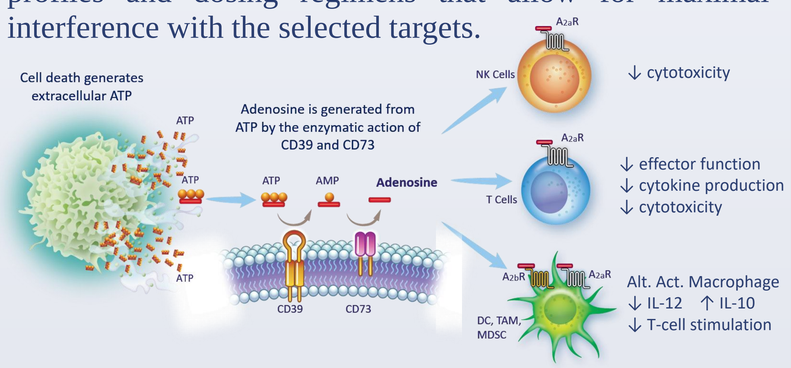
Background

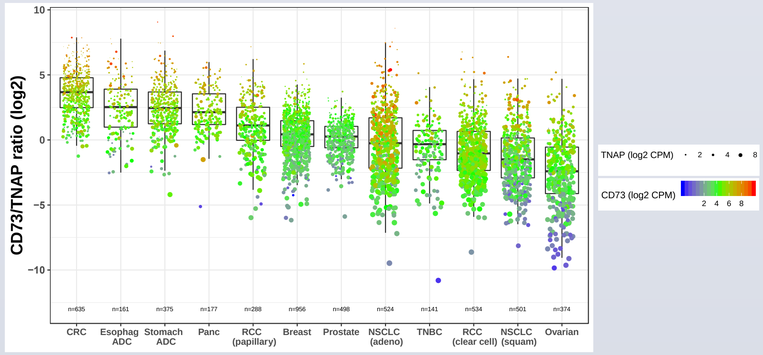
High intra-tumor adenosine concentrations are prevalent and highly suppressive of an effective anti-tumor immune response. This presents multiple therapeutic opportunities, either by preventing extracellular adenosine generation (inhibition of CD73 enzyme) or by blocking adenosine receptors ($A_{2a}R$ and $A_{2b}R$). Translating these therapeutic hypotheses into clinical benefit requires careful selection of tumor types and individual patients most likely to respond to a particular mechanism of action. Equally important is identification of drug candidates with optimal activity profiles and dosing regimens that allow for maximal interference with the selected targets.



Results

Tumor Selection Based on CD73 & TNAP Expression

Like CD73, tissue-non-specific alkaline phosphatase (TNAP) can also convert extracellular AMP into adenosine. The efficacy of A_2R antagonists should be independent of the source of adenosine, while CD73 inhibitors may be more effective in CD73-high/TNAP-low tumors.

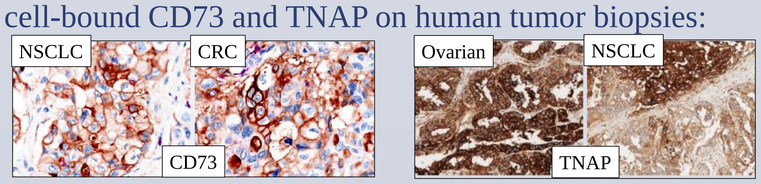


CD73 and TNAP expression were derived from RNAseq in the TCGA database. (See also DiRenzo et al., Abs.#10513, this meeting).

Adenosine Fingerprint Assessment

Adenosine Fingerprint of a given tumor sample is defined by mRNA levels of the enzymes involved in the generation (CD39, CD73, CD38, CD203a, TNAP) and destruction (CD26, ADA) of adenosine.

IHC Methods have been developed to detect and quantitate cell-bound CD73 and TNAP on human tumor biopsies:



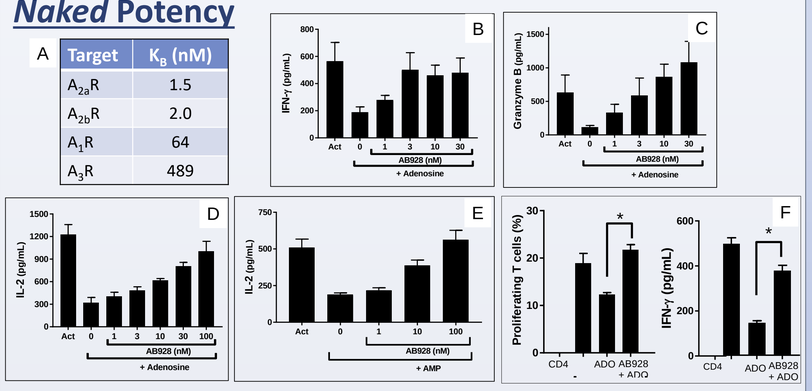
Biochemical Methods have been developed to quantitate the concentration of soluble CD73 in patient plasma, as well as total "AMP-ase" enzymatic activity (reflective of soluble CD73 and TNAP) in patient serum.

(For more details, see: DiRenzo et al, Abs.#10513, this meeting).

AB928 ($A_{2a}R/A_{2b}R$ Antagonist)

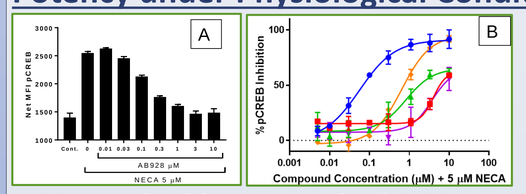
Naked Potency

Target	K_B (nM)
$A_{2a}R$	1.5
$A_{2b}R$	2.0
A_1R	64
A_3R	489



Functional potency and selectivity of AB928 was determined using adenosine receptor over-expressing CHO cell lines (A). AB928 restored CD8 T cell IFN-γ (B) and Granzyme B (C) production in the presence of 6 µM adenosine (representitave data shown; n = 9 donors). AB928 restored CD4 T cell IL-2 production in the presence of 6 µM adenosine (D) and 6 µM AMP (E) (representative data shown, n = 4 donors). AB928 restored normal dendritic cell maturation and activation in the presence of 10 µM adenosine, resulting in significantly increased T cell proliferation and cytokine release in MLR (F).

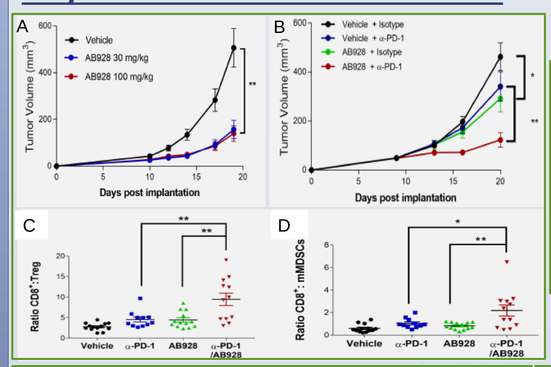
Potency under Physiological Conditions

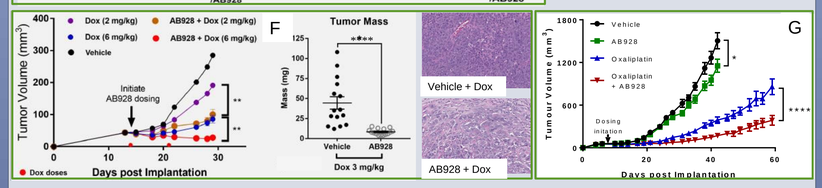


Compound	IC_{50} (nM)
AB928	80
CPI-444	~10,000
AZD 4635	2,600
PBF-509	~10,000
Preladenant	785

AB928 potently inhibits NECA-mediated CREB activation in human blood CD8 T cells (A). The potency of various clinical-stage adenosine receptor antagonists was compared from inhibition of NECA-mediated CREB phosphorylation in blood CD8 T cells (B).

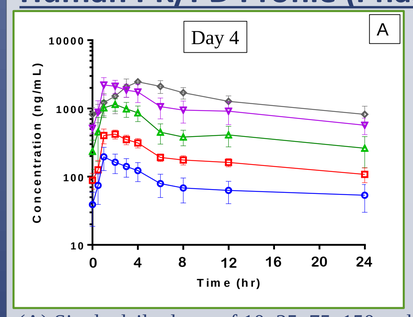
PK / PD in Mouse Tumor Models





AB928 inhibits growth of B16F10 melanoma in C57BL/6 mice, as single agent (A) and in combination with α-PD-1 mAb (B-D). Combination treatment resulted in increased ratios of effector-to-suppressive tumor-infiltrating leukocytes (C, D). AB928 inhibits adenosine receptor-mediated increases in pCREB in mouse blood (E). AB928, as single agent and in combination with doxorubicin (F) or oxaliplatin (G), inhibits the growth of AT3 breast tumors in C57BL/6 mice. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001

Human PK/PD Profile (Phase 1 Healthy Volunteers)



B

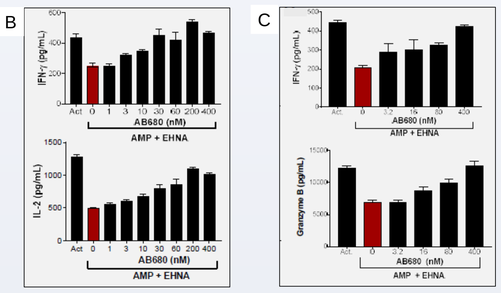
% Inhibition of 5 µM NECA	2-hr Post-Dose	24-hr Post-Dose
200 mg (fed)	≥ 90%	≥ 90%
150 mg	100%	≥ 90%
75 mg	100%	76%
25 mg	82%	51%
10 mg	51%	12%

(A) Single daily doses of 10, 25, 75, 150 and 200 mg were administered for 4 consecutive days. Steady-state plasma levels of AB928 were determined on Day 4; half life ~ 20 hrs. (B) Blood CD8 T-cell CREB phosphorylation in response to exogenous 5 µM NECA was determined by phospho-flow cytometry.

AB680 (CD73 Inhibitor)

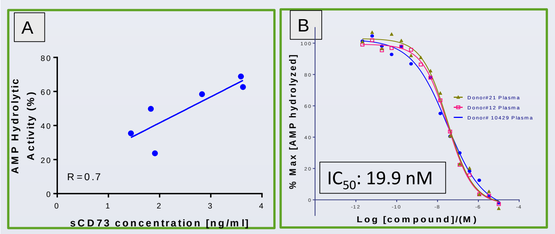
Naked Potency

	System	IC_{50} (nM)
Biochem	Rec. hCD73	0.032
Cell-Based	CHO.hCD73	0.070
	CD8+ T cells	0.008
	CD4+ T cells	0.014



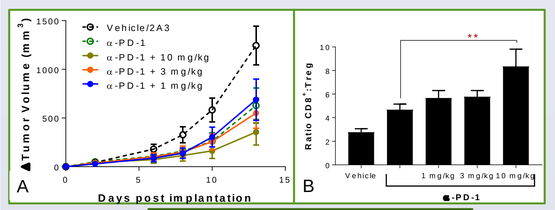
The potency of AB680 was determined using the Malachite Green Assay (A). Human CD4+ T cells (B) and CD8+ T cells (C) were isolated and activated (αCD3/αCD28) in the presence of AMP + EHNA (ADA inhibitor). Dose-dependent rescue of CD4+ and CD8+ T cell activation was observed in the presence of exogenous AB680.

Potency under Physiological Conditions

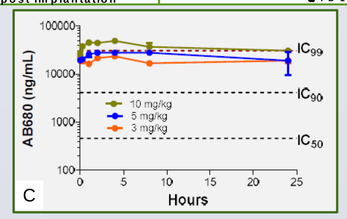


(A) Soluble CD73 in human serum was quantified by ELISA. AMP degradation was measured with AMP-Glo assay. Each dot represents one independent donor.
(B) Potency of AB680 in human plasma was determined by measuring conversion of $^{13}C_5$-AMP to $^{13}C_5$-adenosine by LC/MS/MS (n = 3 donors).

PK / PD in Mouse Tumor Models



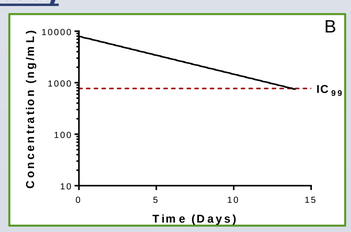
Combined efficacy of AB680 with α-PD-1 was tested in B16F10 tumors using *in life* tumor measurements (A) and CD8-to-Treg ratio in tumor infiltrating lymphocytes (B). Dosing was initiated when tumor volume reached ~50 mm^3. **p < 0.01.



(C) 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.**

Human PK/PD Profile (Projected)

A.	Clearance (L/h/Kg)	V_{ss} (L/Kg)	Half Life
Human	0.0012	0.17	98 h (~4 days)



Predicted human PK parameters were derived by allometric scaling from non-human PK parameters. Vss prediction was determine by the Øie-Tozer method (A).
The predicted human plasma profile shown (B) was determined assuming 89 mg intravenous infusion over the course of 1 hour, resulting in 2-week trough concentration of 772 ng/mL (approximately equal to the IC_{99} of AB680 in human serum).

Conclusions

The totality of the data for AB928 and AB680 (both of which are in clinical development) indicate that 100-150 mg once-daily oral doses of AB928 and 50-100 mg intravenous AB680 every ~2 weeks should be explored in tumor types that either rely on multiple pathways for adenosine generation (AB928) or those that primarily utilize CD73 for that purpose (AB680).

Clinical Development Status & Plans

- AB928 is being evaluated in various combination trials, including with Doxil® (TNBC, Ovarian), FOLFOX (CRC, GE), and α-PD-1 ± Carbo/Pem (NSCLC, Other). (See Abstracts # 10688, 10700, 10706 & 10711, this meeting).
- AB680 is currently being evaluated in a healthy volunteer Phase 1 study.