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$_2$R and A$_3$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$_2$R antagonists should be independent of the source of adenosine, while CD73 inhibitors may be more effective in CD73-high/TNAP-low tumors.

**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:

- **NSCLC**
- **CRC**
- **Ovarian**
- **NSCLC**
- **TNAP**

**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).

**Human PK/PD Profile (Phase 1 Healthy Volunteers)**

AB928 inhibits growth of B16F10 melanoma in C57BL/6 mice, as single agent (A) and in combination with α-PD-1 (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.

**Potency under Physiological Conditions**

Potency of AB928 was determined using adenosine receptor over-expressing CHO cell lines (A). AB928 inhibited CD8 T cell IFN-γ (B) and Granzyme B (C) production in the presence of 6 μM adenosine (representative 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**

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 (representative 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).

**AB928 (A$_2$R/A$_3$R Antagonist)**

**AB680 (CD73 Inhibitor)**

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 (tCD3/ntCD28) 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 $^3$H-AMP to $^3$H-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 life tumor measurements (A) and CD8-to-Treg ratio in tumor infiltrating lymphocytes (B). Dosing was initiated when tumor volume reached ~50 mm$^3$.

(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)**

Predicted human PK parameters were derived by allometric scaling from non-human PK parameters. Vss prediction was determined by the Wee-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 0.72 ng/mL (approximately equal to the IC$_{50}$ 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 Dooxi® (TNBC, Ovarian), POLFOX (CRC, GE), and α-PD-1 ± Carbo/Pem (NSCLC, Other).
- AB680 is currently being evaluated in a healthy volunteer Phase 1 study.