**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 (A2aR and A2bR). 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 A2R 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:

**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 (A2aR/A2bR Antagonist)**
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 Granulocyte Macrophage Colony-Stimulating Factor (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**
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).

**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 (CD3/antiCD28) in the presence of AMP + EHNNA (ADA inhibitor). Dose-dependent rescue of CD4+ and CD8+ T cell activation was observed in the presence of exogenous AB680.

**Potency under Physiological Conditions**

**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 mm³.

**Human PK/PD Profile (Projected)**
Predicted human PK parameters were derived by allometric scaling from non-human PK parameters. Vss prediction was determine by the Ode-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 IC50 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), POLFOX (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.

**Abbreviations**
ADC, Adenosine Deaminase; AMP, Adenosine Monophosphate; ATP, Adenosine Triphosphate; CD73, Tissue-non-specific alkaline phosphatase; CD39, Adenosine deaminase; CD8, T cell; CD4, T cell; CREB, Cyclic AMP Response Element Binding Protein; ELISA, Enzyme-Linked Immunosorbent Assay; IC50, Half Maximal Effective Concentration; IC50, Half Maximal Effective Concentration; IFN-γ, Interferon-gamma; IL-2, Interleukin-2; NECA, Nucleoside 5’-Monophosphate; TNAP, Tissue-nonspecific alkaline phosphatase; TNF, Tumor Necrosis Factor; Vss, Vascular Systemic Space; IC50, Half Maximal Effective Concentration.