pCREB in Human Whole Blood: The inhibition of A2A-mediated effects by AB928 was determined in blood samples from the Phase 1 study by the decreased phosphorylation of CREB following stimulation ex vivo with the adenine receptor agonist NECAT.

At each study point, levels of pCREB were assessed by flow cytometry both in the absence and following ex vivo stimulation with 1, 5 and 10 µM NECA. In order to capture the antagonism of AB928 on both the A2A and A2B receptors, we assessed levels of pCREB on blood CD8+ cells and CD14+ monocytes.

**RESULTS**

AB928 PK Profile Supports Once Daily Dosing at Steady State

**Biological/ PK analysis:** Plasma samples were prepared by a protein precipitation extraction procedure. The analysis, AB928, was column standard. Of AB928, were extracted from plasma with acetonitrile/methanol (80/10, v/v). Concentrations of AB928 in the extracts were determined by LC-MS/MS. Descriptive pharmacokinetic parameters were obtained by a standard non-compartmental analysis from the plasma concentration-time curves using Phoenix WinNonlin v6.3 or higher (Certara, Princeton, NJ).

**Multi-color Phospho-flow Cytometry:** Monoclonal antibodies to cell type specific markers including fluorochrome labelled anti-pCREB (Celsis AMP Response Element Binding Protein) antibody were used to identify inhibition of CREB phosphorylation in plasde and doses subjects.

**NECA-induced pCREB Increases in Peripheral Immune Cells Are Blocked by AB928**

- **NECA-induced pCREB Increases in Peripheral Immune Cells Are Blocked by AB928**
- **AB928 (150 mg qd) provides maximal Inhibition of A2A, R-Mediated pCREB Increases on CD8+ Cells at Steady State**

**Rationale for NECA Concentration Selection:** Previous experiments conducted in vitro indicate that NECA is at least 20 times more potent than adenine as an inhibitor of CREB phosphorylation in human blood, thus, our PD analysis is focused on the 5 µM NECA stimulation under physiologically relevant conditions. 5 µM NECA provided maximal induction of pCREB. We believe this provides receptor inhibition data that are comparable to what we might expect from a 100 µM intra-tumoral adenine. AB928 displays ~100% penetration of mouse tumors (plasma tumor AUC) (~3 [Walters et al, Abstract No. 3158 at this meeting].

**PK/PD Correlation in AB928 Dosed Subjects**

- MNECA: PK/PD Correlation in AB928 Dosed Subjects
  - MNECA: PK/PD Correlation in AB928 Dosed Subjects
  - MNECA: PK/PD Correlation in AB928 Dosed Subjects
  - MNECA: PK/PD Correlation in AB928 Dosed Subjects

**Phase 1 Healthy Volunteer Study:** This Phase 1 healthy volunteer study has single ascending dose (SAD) and multiple ascending dose (MAD) arms. In the SAD arm, we are evaluating single doses of 10, 25, 50, 100, and 200 mg administered for 4 consecutive days. As of the data cut-off date (DCO) of 3/30/18, all doses up to 150 mg in the SAD and MAD arms have been completed. The study is ongoing and remains blinded; no trends have been observed on physiological parameters potentially sensitive to adenine inhibition and there have been no safety events preventing escalation to higher cohorts.

**METHODS**

- **Clinical Pharmacokinetic-Pharmacodynamic Relationship for AB928, a Dual Antagonist of the A2A and A2B Adenosine Receptors**
  - **Clinical Pharmacokinetic-Pharmacodynamic Relationship for AB928, a Dual Antagonist of the A2A and A2B Adenosine Receptors**
  - **Clinical Pharmacokinetic-Pharmacodynamic Relationship for AB928, a Dual Antagonist of the A2A and A2B Adenosine Receptors**
  - **Clinical Pharmacokinetic-Pharmacodynamic Relationship for AB928, a Dual Antagonist of the A2A and A2B Adenosine Receptors**

**CONCLUSIONS**

- **AB928 has demonstrated an excellent oral PK profile in human subjects, consistent with once-daily dosing.**
- **Dose levels of AB928 have been identified that provide maximal adenine receptor coverage in the brain.**
- At these dose levels, AB928 has been shown to be safe and well-tolerated and no effects have been noted on physiological parameters generally associated with adenine inhibition.
- **Assessing CREB levels on whole blood CD8+ T cells following ex vivo stimulation with 5 µM NECA provides an excellent measure of the PD effects of AB928 in dosed subjects.**
- **The clear PK/PD correlation obtained for AB928 in the ongoing Phase 2 study in healthy volunteers will be used to guide dose selection for future combination studies in oncology, several of which are expected to be initiated later this year.**

We also wish to thank PIA Health Sciences in the Netherlands for assistance with the Phase 1 study, Dr. Richard Spriggs, Tien-Vanh Vu, Patricia Weyman, F Rafidye, Bayram Bangerter and Joerg Steiger.