The tumor microenvironment (TME) contains high levels of immunosuppressive adenosine (ADO), which activates the A2aR and A3R receptors on immune cells, leading to an ineffective anti-tumor response. Ecto-5'-nucleotidase (CD73) and tissue non-specific alkaline phosphatase (TNAP) are primarily responsible for the conversion of extracellular adenosine mono-phosphate (AMP) to ADO and exhibit both membrane-bound and secreted forms. We have previously shown that AB928, a dual A2aR/A3R antagonist, rescues the immune suppressive effects of ADO in experimental tumor models. Herein, we describe the development of assays to measure the expression and activity of adenosine-generating enzymes in human tumor samples and peripheral blood. These assays are being used to define an "adenosine fingerprint" to identify tumor types and patients most sensitive to adenosine inhibition by AB928.

CD73 ELISA: Circulating levels of CD73 were quantified in matched serum and plasma samples from healthy donors and cancer patient samples with an in-house ELISA. Validation studies were performed to correlate CD73 levels between serum and plasma and to ensure acceptable assay parallelism and inter-assay variation.

AMPase Activity: AMP hydrolytic activity in serum from healthy volunteers or patients with solid tissue tumors was assessed using the AMP Glo™ (Promega) assay. CD73 and TNAP inhibitor cocktails were used to assess the relative contribution of each enzyme to AMPase activity.

Gene Expression: Expression of CD73 (WTSE) and TNAP (ALPL) on select tumor types were derived from RNAseq in The Cancer Genome Atlas (TCGA) database. Data is displayed as log2 transformed expression of counts per million.

Histology: Immunohistochemistry (IHC) was performed using CD73 (Cell Signaling, D7F9A) and TNAP (Sino Biological, R0464) on formalin fixed paraffin embedded (FFPE) human tissue. QuPath software was used for quantification and H-score (high intensity staining area) + 2x (medium intensity staining area) + 1x (low intensity staining area).

Results

AB928 Inhibits A2aR Activation Following Dosing with ≥75 mg in Healthy Volunteers and Cancer Subjects

Conclusions

- Robust assays have been developed to measure the peripheral levels and activity of the adenosine-generating enzymes, CD73.
- Immunohistochemical methods have been developed to measure and quantify adenosine-generating enzymes CD73 and TNAP in human tumor tissue.
- These assays are being implemented to assess the adenosine generating potential of subjects in clinical studies with AB928.
- AB928 is currently in clinical trials in combination with standard of care chemotherapeutics and an anti-PD-1 immunotherapeutic.

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