MEASURING SOLUBLE CD73 ACTIVITY IN HIGH CONCENTRATIONS OF HUMAN PLASMA TO ASSESS PHARMACODYNAMICS OF CD73 INHIBITORS

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Background Inhibiting ecto-5’-nucleotidase (CD73) to reduce immunosuppressive adenosine in the tumor microenvironments is an anti-tumor strategy currently explored in clinical trials. Measuring soluble CD73 (sCD73) activity in plasma to evaluate pharmacodynamics of CD73 inhibitors is appealing. Quantifying phosphate in plasma after adding exogenous adenosine monophosphate (AMP) can be used to determine sCD73 activity. Maintaining high plasma concentration to prevent dilution of endogenous sCD73 when quantifying its activity is desirable. High protein concentrations in plasma, however, can affect accurate phosphate quantitation. By precipitating plasma proteins prior to phosphate quantitation, we developed and qualified a method to determine sCD73 activity in 95% plasma.

Methods Platelet poor heparinized plasma (PPP), AMP, tissue nonspecific alkaline phosphatase inhibitor (TNAPi), recombinant CD73, malachite green, adenosine 5’-(ε, β-methylene)diphosphate (APCP), and recombinant alkaline phosphatase (ALP) were procured commercially. sCD73 concentrations were measured by ELISA and total protein concentration was quantified by BCA. sCD73 activity was measured by combining PPP, TNAPi, and AMP at 37°C. Reactions were terminated with trichloroacetic acid (TCA) at various timepoints to generate a kinetic readout. After protein precipitation, phosphate concentrations were measured by malachite green and enzymatic rates calculated as change in free phosphate concentration per minute.

Results Incubating TCA-terminated reaction mixtures at 4°C for ≥ 3 hours reduced protein in supernatants to below lower limits of quantitation and eliminated interference in phosphate detection. Plasma sCD73 activity was dependent on AMP concentrations (Km = 612 μM), proportional to sCD73 in the sample and could be fully inhibited by APCP 500 μM TNAPi, an inhibitor of non-CD73 AMPase activity, fully blocked 670 IU/L of recombinant human ALP activity. sCD73 activity in PPP from colorectal carcinoma (CRC) patients was higher (p= 0.0028) than in healthy volunteers (HV). sCD73 activity in some individuals with gastric cancer (GC), non-small cell lung cancer (NSCLC), and head and neck squamous cell carcinoma; TNBC, triple-negative breast cancer.

Abstracts
Plasma sCD73 activity assay characterization and fit-for-purpose qualification. Abbreviations: LOD, limit of detection; LLOQ, lower limit of quantitation; ULOQ, upper limit of quantitation; APCP, adenosine 5'- (α, β-methylene) diphosphate.

Conclusions A method to quantify sCD73 activity in 95% plasma to evaluate pharmacodynamics of CD73 inhibitors in clinical samples was developed and qualified. Plasma sCD73 activity was dependent on AMP concentration and inhibited by APCP. Plasma sCD73 activity was significantly elevated in CRC patients and selected patients with GC, NSCLC, and TNBC and was proportional to sCD73 concentration.

REFERENCE

http://dx.doi.org/10.1136/jitc-2021-SITC2021.032