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.¹ 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'- (α , β -methylene) diphosphate (APCP), and recombinant alkaline phosphatase (ALP) were procured commercially. sCD73 concentrations were measured 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.

Abstract 32 Figure 1a sCD73 activity in HV and cancer patient plasma. sCD73 activity in patient plasma from healthy volunteers and solid tumor indications. Abbreviations: HV, healthy volunteer; CRC, colorectal cancer; NSCLC, non-small cell lung cancer; GC, gastric cancer; HNSCC, head and neck squamous cell carcinoma; TNBC, triple-negative breast cancer.

Abstract 32 Figure 1b sCD73 activity correlates to sCD73 concentration. Correlation of plasma sCD73 activity and free sCD73 concentrations in patient plasma from healthy volunteers and solid tumor indications. Spearman correlation: $r = 0.75$, $p < 0.0001$, $n = 135$. Abbreviations: HV, healthy volunteer; CRC, colorectal cancer; NSCLC, non-small cell lung cancer; GC, gastric cancer; HNSCC, head and neck squamous cell carcinoma; TNBC, triple-negative breast cancer.

Abstract 32 Table 1 Plasma sCD73 activity assay qualification limits

Parameter	Result
Range	1.09 – 133 $\mu\text{M PO}_4/\text{min}$
Limits	LOD: 0.74 $\mu\text{M PO}_4/\text{min}$ LLOQ: 1.09 $\mu\text{M PO}_4/\text{min}$ ULOQ: 133 $\mu\text{M PO}_4/\text{min}$
Precision	Intra-assay: 4.33 – 17.2 %CV Inter-assay: 10.2 – 18.4 %CV
Freeze/Thaw stability (80% - 120% expected)	4 Freeze/Thaw cycles: 90.2% – 108.0%
Parallelism (80% - 120% expected)	92.6% - 112.5%
Specificity (maximum inhibition by APCP)	APCP: 93.7% - 103.1%
Plasma stability	3 hours at room temperature
Phosphate stability	164 hours at 4°C
Longitudinal variation (Day 1 and Day 8)	1.1 – 12.5 %CV

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 ($K_m = 612 \mu\text{M}$), 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 triple-negative breast cancer (TNBC) were also numerically higher than in healthy volunteers (figure 1a). sCD73 activity was correlated to sCD73 concentrations in these samples (figure 1b). The method was qualified for use in clinical studies (table 1).

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

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