timsTOF HT IMPROVES PROTEIN IDENTIFICATION AND QUANTITATIVE REPRODUCIBILITY FOR DEEP UNBIASED PLASMA PROTEIN BIOMARKER DISCOVERY

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INTRODUCTION

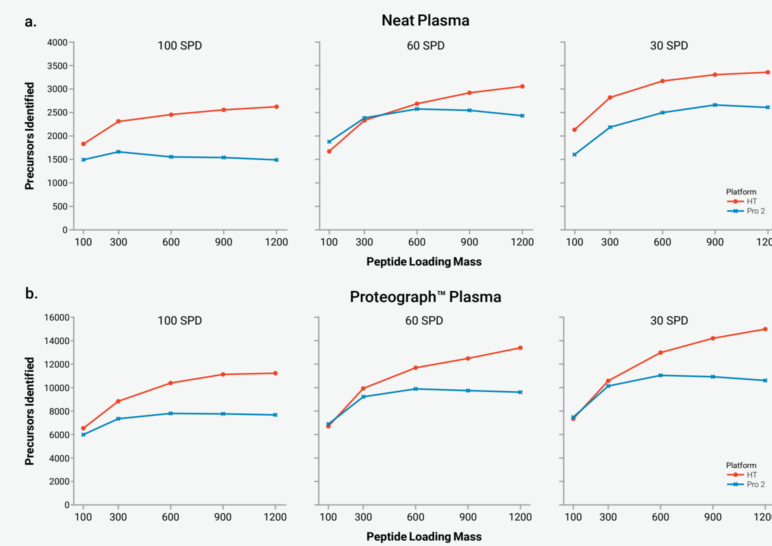
- Protein biomarkers measured in liquid biopsies offer a non-invasive approach for disease diagnosis
- Nevertheless, technical variability associated with mass spectrometry analysis of complex biological samples, such as plasma, presents a significant challenge for obtaining reproducible results across large patient cohorts
- The newly developed timsTOF HT, equipped with a 4th-generation trapped ion mobility spectrometry (TIMS) analyzer and with improved digitizer speed and resolution, holds potential to advance the field of plasma biomarker discovery

OBJECTIVE

- Evaluate qualitative and quantitative performance of timsTOF Pro 2 and HT across a wide range of plasma peptide loading masses and liquid chromatography (LC) gradients for neat and Proteograph™ (PG)-processed plasma
- Identify the optimal sample processing, peptide loading mass, and LC gradient for deep profiling and reproducible quantitation of proteins in plasma
- Demonstrate increased performance of timsTOF HT vs Pro 2 in detecting plasma protein biomarkers within a cancer vs control study

RESULTS

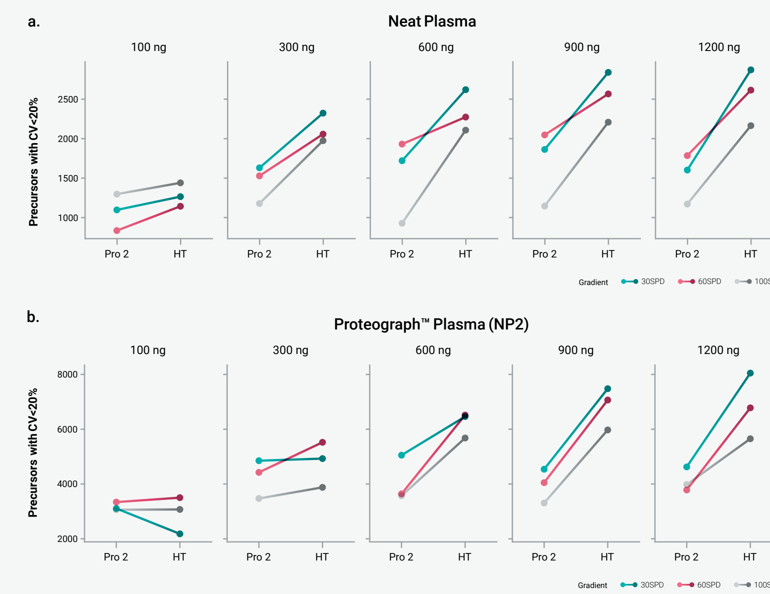
FIGURE 2. timsTOF HT qualitative performance exceeded timsTOF Pro 2 across a wide range of plasma peptide loading masses and LC gradients.



Number of precursors uniquely detected in neat (top) and PG plasma (bottom; NP1-3,5 panel). Data for NP4 not plotted due to insufficient yield for 1200 ng peptide load.
SPD, samples per day.

- Compared to timsTOF Pro 2, timsTOF HT increased precursors identified by up to 76% and 46% in neat and PG plasma, respectively, with over 4.5-fold more precursors detected in PG compared to neat plasma
- The reduction in precursors identified at higher loading masses in timsTOF Pro 2, but not timsTOF HT, suggests saturation at the mass spectrometry level

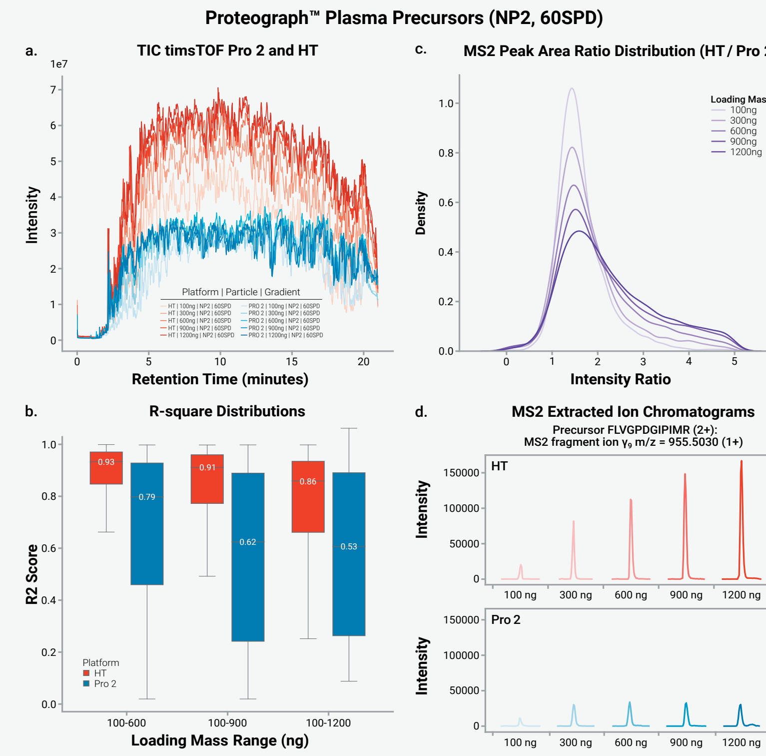
FIGURE 3. timsTOF HT had enhanced reproducibility compared to timsTOF Pro 2.



Quantitation: MS2-based peak area of triplicate measurement.
SPD, samples per day.

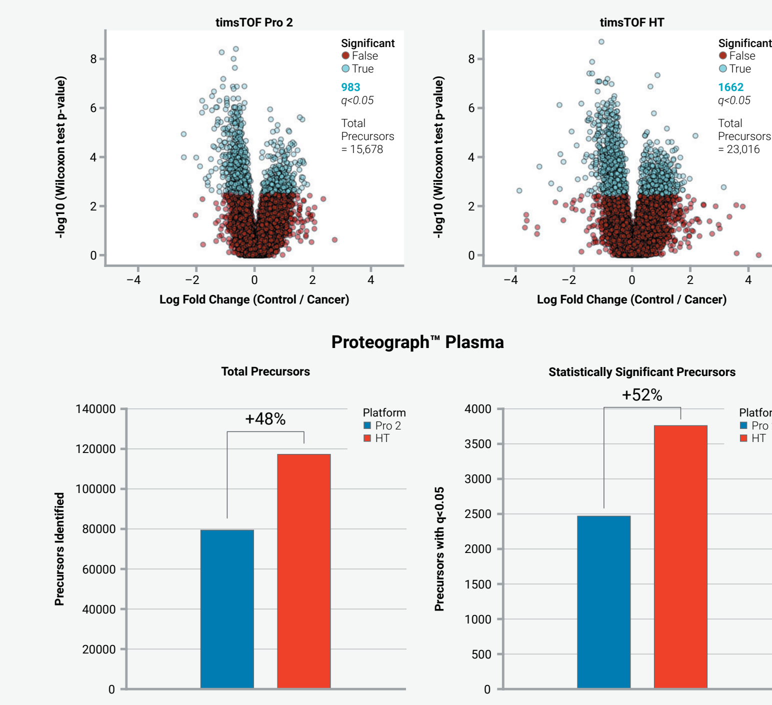
- The timsTOF HT increased the number of reproducibly quantified precursors (CV<20% of triplicate measurements) compared to timsTOF Pro 2 for up to 127% in neat and 81% in PG plasma (NP2) across different LC gradients
- The quantitative reproducibility of the timsTOF HT over Pro 2 improved significantly as the loading mass increased

FIGURE 4. timsTOF HT provided superior quantitative linear range compared to timsTOF Pro 2.



(a) Representative TIC of a single PG NP2 replicate load of 100-1200 ng at 60 SPD gradient. (b) R-square distribution for the common quantified precursors in triplicate measurement of each peptide loading mass within the 100-1200 ng range for PG NP2 at 60 SPD gradient for timsTOF HT (n=4,256) and timsTOF Pro 2 (n=3,331). (c) Distribution of precursor MS2 peak area (triplicate average) ratios quantified in timsTOF HT vs Pro 2. (d) Extracted ion chromatogram of MS2 fragment ion (y_6) for selected precursor FLVGPDGIPIMR (2+) within the 3rd quartile of total precursor intensity range.
TIC, Total Ion Chromatograph; NP, nanoparticle.

FIGURE 5. The qualitative and quantitative improvements of timsTOF HT allowed for higher sensitivity and reproducibility of cancer biomarkers detected in plasma.



- In a control (n=20) vs cancer (n=20) study, improved sensitivity and reproducibility of timsTOF HT resulted in 48% more precursors compared to timsTOF Pro 2, which translated to 52% more statistically significant features across 5 nanoparticles

CONCLUSIONS

- The timsTOF HT qualitatively and quantitatively outperformed timsTOF Pro 2
- Proteograph-processed plasma analyzed at a 600-1200 ng peptide load on an EvosepOne-timsTOF HT enabled deep plasma proteome profiling with exceptional quantitative reproducibility and linearity
- Our case-control study suggests that timsTOF HT has superior performance for detecting plasma disease biomarkers at scale compared to timsTOF Pro 2

REFERENCES

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DISCLOSURES

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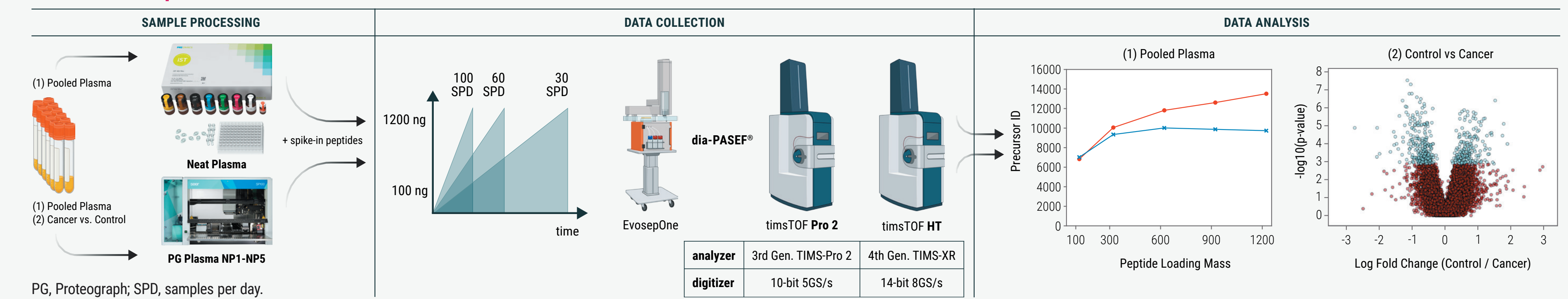
METHODS

- Control pooled human plasma (BioIVT) was processed using Proteograph™ workflow with Proteograph Assay (PG plasma; Seer Inc.) and filter-based digestion by PreOmics iST HT 192x kit (neat plasma; PreOmics Inc.)
- Individual plasma from 20 cancer patients and 20 controls underwent PG processing only
- Control pooled plasma peptide masses 100-1200 ng (neat; PG nanoparticle [NP]1-3, 5) or 100-900 ng (PG NP4) were separated on PepSep columns of 8 cm (150 µm x 8 cm, 1.5 µm) for 100 and 60 samples per day (SPD) and 15 cm (150 µm x 15 cm, 1.9 µm) for 30 SPD, followed by acquisition in triplicates
- Individual plasma samples were analyzed with 60 SPD gradient at 600 ng (NP1-3,5) or 300 ng (NP4) peptide load
- All samples were acquired on an EvosepOne coupled to timsTOF Pro 2 and timsTOF HT under dia-PASEF® mode and processed via DIA-NN v1.8.1 with proprietary spectral library
- Only precursors commonly identified in control pooled plasma triplicates across the same sample (neat; PG NP1-5), loading mass, and LC condition were considered
- For individual samples, precursor identification cutoff was set to a minimum 25% in cancer or control groups
- Data were visualized in Python and schematics were created with BioRender.com

FIGURE 1. Experimental workflow.



PG, Proteograph; SPD, samples per day.