TRANSLATION OF PROTEOGRAPH XT NANOPARTICLE-ENRICHED PLASMA PROTEOMIC FEATURES TO A TARGETED ASSAY

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INTRODUCTION

- Measuring biomarkers in plasma provides a highly accessible and minimally invasive approach for disease diagnostics, including early cancer detection
- Untargeted proteomic mass spectrometry (MS) holds great potential in plasma biomarker discovery; however, translation into targeted MS assays for downstream validation can be time-consuming and labor-intensive
- The recent development of the Thermo Scientific™ Stellar™ Mass Spectrometer (Stellar MS) enables the rapid translation of untargeted biomarker discovery results into targeted MS assays for downstream validation
- We developed multiple assays with different throughputs and peptides to understand the capabilities of the Stellar MS for verification of biomarkers from two large biomarker discovery studies with 2513 and 3000 subjects each
- A 60-minute quantitative targeted assay of ~1960 targets (~3920 peptides) was developed with the Stellar MS in 3 days; we are currently applying this targeted assay for biomarker verification and validation with biological cohorts

OBJECTIVE

- Demonstrate the capabilities of the Stellar MS, a new hybrid nominal mass spectrometer, to rapidly translate results from a large scale biomarker study into a large scale, robust and sensitive targeted assay

METHODS

Sample Preparation and Data Acquisition (Figure 1)

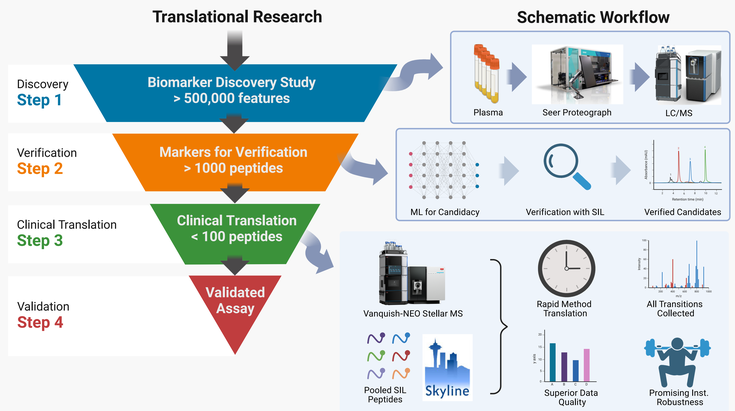
- 40 aliquots of pooled male and female plasma standard were processed on the Proteograph™ Product Suite XT (Seer Inc.) to generate Proteograph plasma samples
- Neat plasma digest samples were generated by applying S-trap digestion to several aliquots of pooled plasma standards
- A total of 2163 stable isotopic label (SIL) peptides (Biosynth, Biognosys, SciTides, Genscript, and ElimBio) were prepared according to the manufacturer's guidance; P100, P800, and P2000 versions were used for quantitative targeted assays
  - Neat plasma digest samples were used as background matrix for the P100 assay and Proteograph plasma samples were used as background matrix for the P800 and P2000 assays
  - 2163 SIL peptides were used to develop the P2000 assay; the final version of P2000 assay contained 1960 targets
  - All mixtures were diluted with a buffer of 0.1% formic acid in 95% water/5% acetonitrile
- All prepared samples were subjected to either Data Independent Acquisition (DIA) or Parallel Reaction Monitoring (PRM) collection on a Thermo Scientific Vanquish NEO liquid chromatography (LC) system coupled to the Stellar MS for targeted assay construction
- Skyline (v23.1) and PRM conductor (a new Skyline plug-in) were used to accelerate PRM assay construction and evaluate data quality

Study Data Validation

- Plasma samples collected as part of a proof-of-concept study (23 subjects with cancer and 21 non-cancer controls) were processed as described above and run on the NEO-Stellar system using the P2000 assay

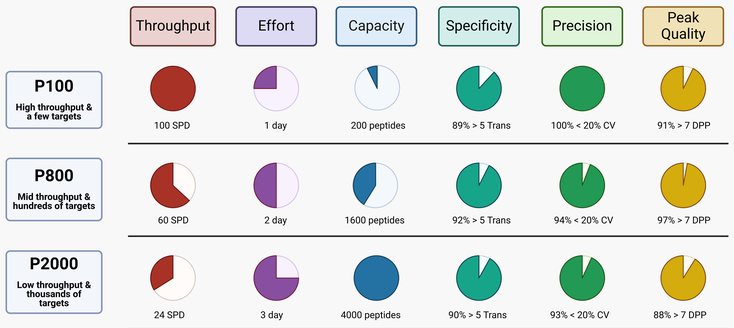
RESULTS

FIGURE 1. Accelerated Clinical Translation of Biomarkers.



Inst, Instrument; LC/MS, liquid chromatography/mass spectrometry; ML, machine learning; SIL, stable isotopic label.

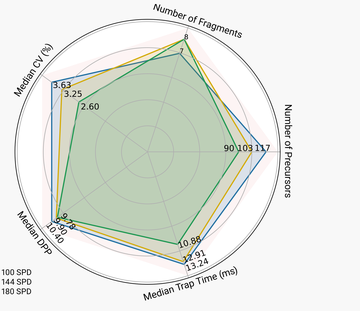
FIGURE 2. Comparison of 3 Targeted Assays Developed on the Stellar MS.



CV, coefficient of variation; DPP, data points per peak; SPD, samples per day; Trans, transitions.
Proteograph XT nanoparticle Well #1 data presented.

- All 3 assays were developed using ≤ 3 days of effort and demonstrated the desired quantitative performance and assay specificity (**Figure 2**)

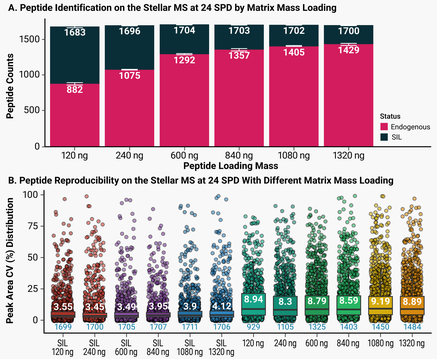
FIGURE 3. The Stellar MS Enables Large (> 100 peptides) Quantitative Targeted Assays at High Throughput.



CV, coefficient of variation; DPP, data points per peak; ms, milliseconds; SPD, samples per day.

- The P100 assay was evaluated at 3 different throughputs: 100 samples per day (SPD), 144 SPD, and 180 SPD (**Figure 3**)
  - While quantitative performance was maintained at all 3 SPD rates, the number of measured targets dropped with increased throughput

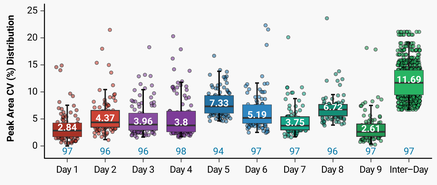
FIGURE 4. Proteograph-Processed Plasma Loading Mass Optimization for a Targeted Assay.



CV, coefficient of variation; SIL, stable isotopic label; SPD, samples per day.
Proteograph XT nanoparticle Well #1 data presented.
Median number of peptides identified in each loading mass condition shown in blue.

- 1700 SIL peptides were used to optimize the loading mass prior to developing a targeted assay using all 2100 SIL peptides
  - An increased number of endogenous peptides were identified with increased matrix mass (**Figure 4A**)
  - Quantitative reproducibility was high (coefficient of variation < 10%) across a wide range of loading masses on the Stellar MS (quadruplicate injections) (**Figure 4B**)
- Based on these results, 600 ng matrix was selected as the loading mass for plasma samples

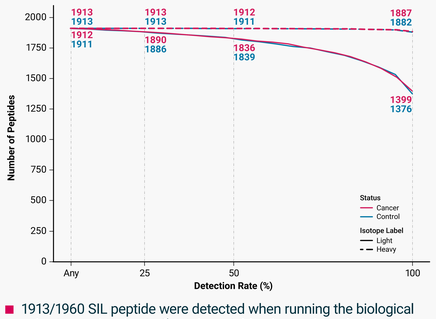
FIGURE 5. The Stellar MS Generated Reproducible Results Over Several Days in a Proof-of-Concept Biomarker Study.



CV, coefficient of variation.
Median number of peptides identified in each loading mass condition shown in blue.

- Data from neat plasma digests spiked with 98 SIL peptides at 100 fmol highlights the robustness and reproducibility of the Stellar MS over many days for plasma proteomics biomarker verification (**Figure 5**)

FIGURE 6. Stable SIL Performance and Full Coverage of Peptides of Interest in Proof-of-Concept Biomarker Study.



- 1913/1960 SIL peptide were detected when running the biological cohort samples due to 1) peaks outside of retention window; and 2) some filters applied for processing these cohort data (**Figure 6**)

CONCLUSIONS

- P100, P800, and P2000 assays, developed using the NEO-Stellar LC/MS system, all displayed excellent quantitative performance and assay specificity and required 1 – 3 days of effort, compared to weeks or months required using traditional methods with a triple quadrupole
- Increasing the throughput of the P100 assay to 100 SPD, 144 SPD, and 180 SPD demonstrated the consistent quantitative capabilities of the Stellar MS at high throughput
- A wide range of Proteograph plasma loading masses were tested, and an optimal matrix loading mass of 600 ng was selected for proteomic biomarker validation
- The high reproducibility (< 12% inter-day CV) and sensitivity of the NEO-Stellar across a proof-of-concept study of 44 subject samples suggests that this LC/MS system could facilitate verification of much larger numbers of putative biomarkers than was previously possible

Disclosures

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