Proteomic Profiling of Prostate Cancer Plasma Specimens Using Proteograph and TIMS Technology

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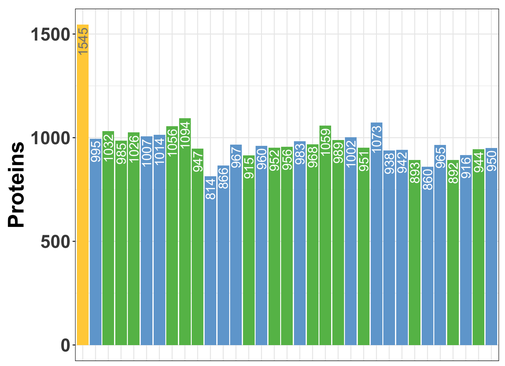
OVERVIEW

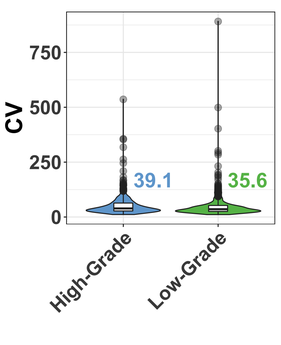
The Seer Proteograph platform affords a unique combination of deep proteomic sampling and study scalability, a breakthrough development for proteomic biomarker discovery studies.

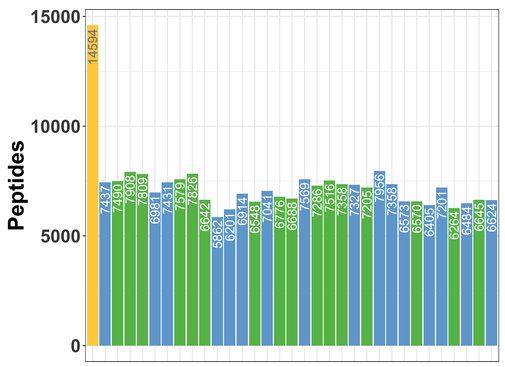
A proof-of-concept pilot study was initiated on 32 prostate cancer plasma specimens retrospectively collected from patients with high and low tumor grades.

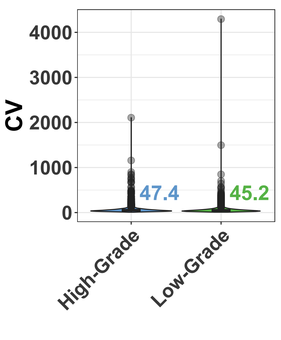
While admittedly underpowered for biomarker discovery, this study provided results motivating multiple scaled biomarker discovery studies currently in progress.

PROTEOMIC COVERAGE





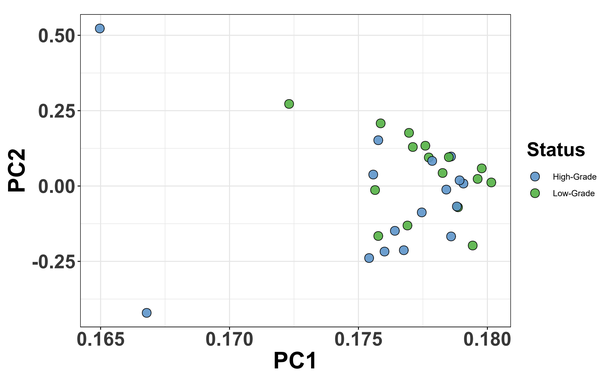




High-Grade Low-Grade Combined

Protein and Peptide Identification Rates and Variability
Protein group identifications per sample are displayed in the top left panel with an average of 947 in high-grade and 960 in low-grade samples. Variation in protein intensities for both groups is shown in the adjacent top right panel with median CV displayed.

Peptide identifications per sample are displayed in the lower left panel with an average of 6960 in high-grade and 7047 in low-grade samples. Variation in peptide intensities for both groups is shown in the adjacent lower right panel along with median CV.



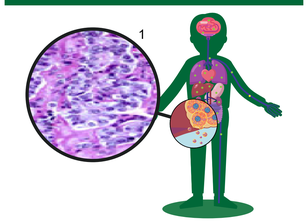
Principal Component Analysis
Samples clustered based on protein intensities with the exception of three potential outliers that were not removed from the overall analysis here.

SPECIMENS AND WORKFLOW

Existing double-spun prostate cancer patient plasma selected from available set in our internal biorepository. A small set (n = 32, 16 per group) were selected as "high" or "low" grade based on several factors and under the consult of Dr. Ryan Kopp.

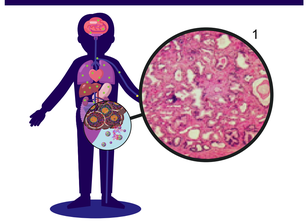
OHSU CEDAR specimen biorepository sourcing from OHSU and VA hospitals

"Low-Grade" Study Arm, n =16



- Benign prostate hyperplasia
- Prostatic intraepithelial neoplasia
- Age range 52-74 (avg. 66)

"High-Grade" Study Arm, n =16

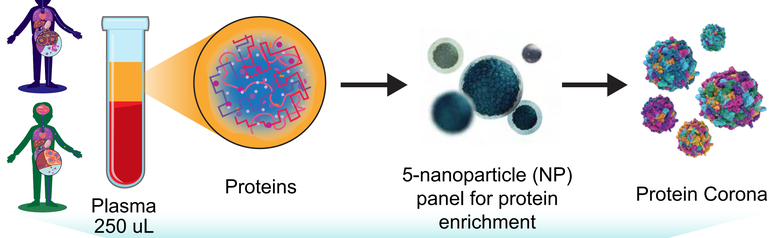


- Adenocarcinoma
- Gleason grade ≥ 4+3
- Age range 56-72 (avg. 67)

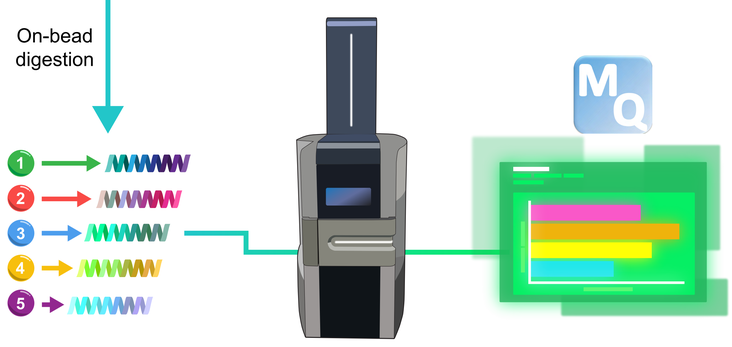
Proteograph™ Product Suite

1 hr setup
7 hr automated workflow

16 experimental samples per Proteograph plate + multiple internal controls

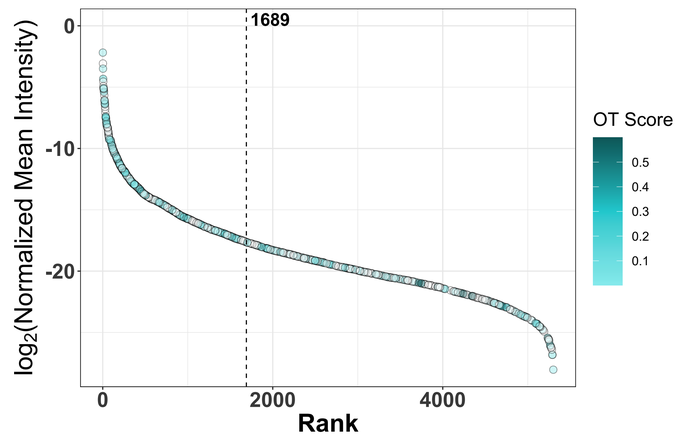


On-bead digestion



Peptide Preparation	timsTOF Pro LC-MS Analysis	Data Analysis
• Manual fluorescence peptide assay (Pierce/ThermoScientific) • Standard mixture of internal peptide standards (PepCal, SCIEX)	• nanoELUTE LC (Bruker) • CaptiveSpray source (Bruker) • Aurora C18 column, 25-cm (IonOpticks) • nanoEase M/Z Symmetry C18 trap (Waters) • DDA-PASEF acquisition mode	• MaxQuant v1.6.17.0 • Reference proteome: *Homo sapiens*, Aug 2019 • Peptide and protein FDR for identification at 1%. • Output analyzed in R.

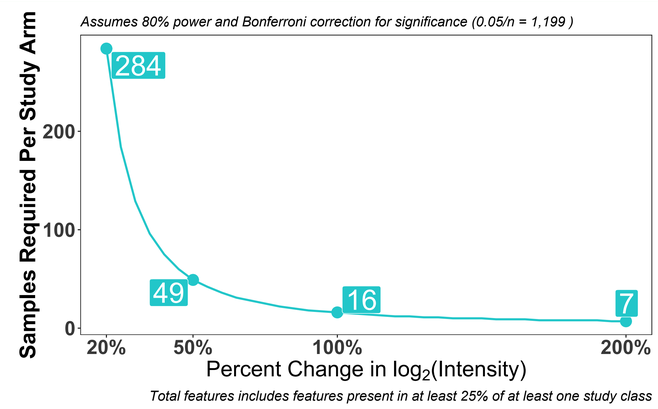
DYNAMIC RANGE



Measurement Depth in Plasma
Identified proteins spanned a wide dynamic range across the plasma proteome as displayed with reference to normalized intensities from Keshishian et al.[2] The mean rank of mapped proteins is displayed as a vertical line.

Proteins previously demonstrated as associated with prostate cancer based on the Open Targets (OT) Platform are colored according to their OT score. Points colored white mapped back to the plasma proteome reference but did not match the OT query.

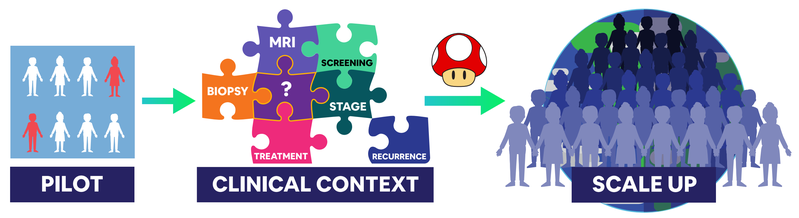
STUDY POWER ESTIMATE



Total features includes features present in at least 25% of at least one study class

Statistical Power Curve and Sample Size Estimation
Relationship between increasing sample number and ability to detect more subtle fold changes across study arms within the context of this assay.

CURRENT WORK



Future work includes highly scaled studies in solid and liquid tumor indications
- Careful study contextualization based on clinical need.
- Large sample numbers for reducing patient-patient variance.
- PRoBE biomarker discovery framework[3] for improved study design.

Evolving improvements in our workflow
- Exploration of alternative specimen types (e.g. serum).
- diaPASEF (data-independent acquisition PASEF) for increased sampling depth, reproducibility, and lower acquisition times.
- Broad survey of analytical column types for optimized blend of sensitivity and ruggedness.
- Post-acquisition processing using a variety of spectral library-based and library-free approaches with DIA-NN[4] and Seer Proteograph Analysis Suite (PAS).

References
1. Nickel, J. Curtis. "Prostate Inflammation and Prostate Cancer: What do I need to know?" January 27, 2018. Accessed Oct 2021. https://grandroundsinurology.com/Prostate-Inflammation-and-Prostate-Cancer/
2. Keshishian, H. et al. Quantitative, multiplexed workflow for deep analysis of human blood plasma and biomarker discovery by mass spectrometry. *Nat. Protoc.* 12, 1683–1701 (2017).
3. Pepe, M. S., Li, C. I., & Feng, Z. Improving the Quality of Biomarker Discovery Research: The Right Samples and Enough of Them. *Cancer Epidemiol Biomarkers Prev.* 24(6), 944-950 (2015).
4. Demichev et al. DIA-NN: neural networks and interference correction enable deep proteome coverage in high throughput. *Nat. Methods.* 17(1), 41-44 (2020).