Advancing Biomarker Discovery: Characterization of the P2 Technology for Plasma Samples for Pharma and Biobank, Use and Application to NSCLC

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ABSTRACT PRESENTATION NUMBER: MP 141

BIOGNOSYS
NEXT GENERATION PROTEOMICS

INTRODUCTION

Plasma is the most widely collected biofluid and an invaluable source of biomarkers. The analysis of plasma using discovery mass spectrometry–based proteomics faces challenges as the 22 most abundant proteins constitute more than 99% of the total protein content. This is hindering the detection of lower abundant proteins, potentially disease-relevant biomarkers. To overcome this, we developed and optimized an enrichment workflow, termed P2 Plasma Enrichment System, based on protein corona formation. Protein corona formation leads to a reduction in dynamic range enabling the detection of lower abundant proteins. We characterized P2 in terms of pre-analytical variation, robustness and quantification. Finally, we applied it to find predictive biomarkers in a multicentric phase II clinical trial (SAKK17/18) in patients with non-small cell lung cancer (NSCLC).

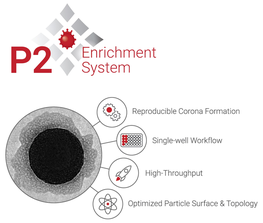

Figure 1 P2 Enrichment System is based on protein corona formation. Using an optimized particle surface and optimal buffer conditions reproducible protein corona is formed. This workflow is single-well and high-throughput. Adapted from Gopinath, 2019, Sci.Rep.[1]

METHODS

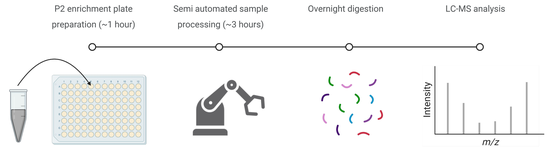

Figure 2 P2 enrichment workflow created with BioRender.com

We performed P2 plasma protein enrichment starting with 100µL of plasma followed by tryptic digestion. Samples were processed in a semi-automated fashion using a KingFisher Flex. We first characterized our workflow robustness over 21 plates (**Figure 3**), labs (**Figure 4**), batches of P2 materials (**Figure 5**) and finally to blood collection methods (**Figure 7**). Further, we prepared and analyzed 95 samples from phase II clinical trial (SAKK17/18) in patients with NSCLC (**Figure 8**). Plasma samples were collected at baseline (n=33) and at two post-dose time points (C1D8, n=31 and C2D1, n=31) (**Figure 9—11**). The samples were acquired in data-independent acquisition (DIA) either on a timsTOF HT (Bruker) or on an Exploris 480 mass spectrometer (Thermo Scientific) equipped with FAIMS Pro. Data analysis was performed using Spectronaut® 19 (Biognosys) using directDIA™.

RESULTS: WORKFLOW CHARACTERIZATION

PLASMA QC SHOWS GREAT STABILITY ACROSS 21 96-WELL PLATES AND 2.5 MONTHS

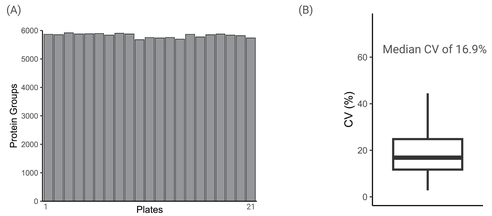

Figure 3 One plasma QC was added to each 96-well plate and analyzed using SN 19 directDIA (**A**) protein groups (PG) identified in each QC (plate) (**B**) Coefficient of variation (CV) % of protein groups across the 21 plates. **There is no drop in protein identified over 2.5 months and the identifications are stable**.

INTERLABORATORY REPRODUCIBILITY: BIOGNOSYS AG, BIOGNOSYS INC AND GOTHENBURG UNIVERSITY

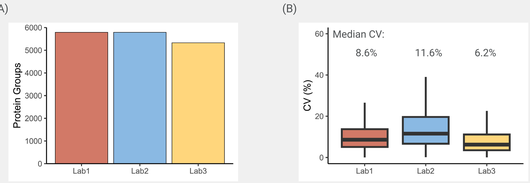

Figure 4 Interlaboratory comparison using the same plasma QC. Samples were prepared independently in each laboratory. Lab 1 is Biognosys AG, Lab 2 is Biognosys Inc. and Lab 3 is the University of Gothenburg. (**A**) Protein groups identified by each laboratory (n=3) (**B**) CV% of protein groups. Note that for Lab 2 the QCs were processed and analyzed on different days. **The P2 workflow is transferable to other labs.**

BATCH TO BATCH REPRODUCIBILITY

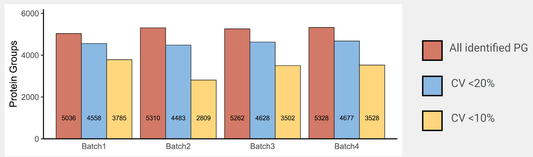

Figure 5 Four batches of the P2 particles were tested in quadruplicate. All batches performed similarly in terms of protein groups identified and CV. **There is very low variability between P2 particles batches.**

LINEARITY AND QUANTITATION WAS EVALUATED USING A PROTEIN SPIKE IN EXPERIMENT

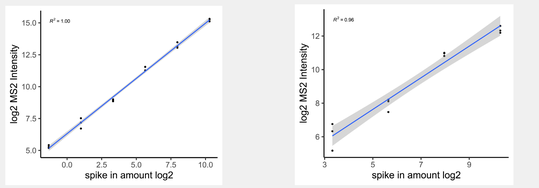

Figure 6 Protein were spiked in plasma 6 different amount 0.4ng, 2ng, 10ng, 50ng, 250ng and 1250ng, prepared in triplicates. (**A**) IL6 and (**B**) is the phosphorylated peptides of Tau p217. **In each experiment the correlation R^2 was >0.95, highlighting the linearity of the enrichment**.

P2 PERFORMANCE ACCROSS BLOOD COLLECTION TYPES

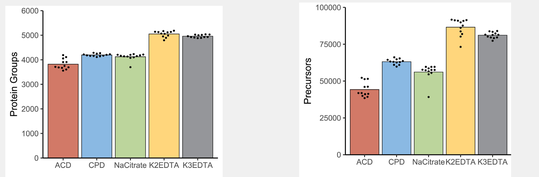

Figure 7 Five different plasma collection methods were evaluated: ACD (Acid Citrate Dextrose), CPD (Citrate Phosphate Dextrose), NaCitrate (Sodium Citrate), K2EDTA and K3EDTA. For each plasma type, samples from 4 match individuals were collected. **Blood collection is a key pre-analytical factor to control for.**

RESULTS: BIOMARKER DISCOVERY

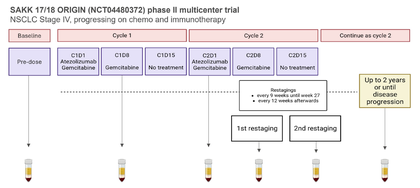

Figure 8 SAKK 17/18 Origin phase II multicentric trail overview.

PLASMA PROTEIN CHANGES DIFFERENTIATE TREATMENT RESPONSE BEFORE AND AFTER DOSING

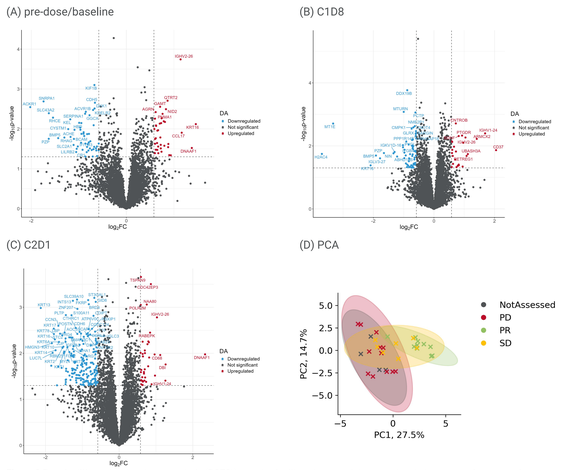

Figure 9 Protein differential abundance analysis (ROTS[2]) reveals significantly up- and downregulated plasma proteins comparing partial response (PR) and progressive disease (PD) at (**A**) pre-dose/baseline; (**B**) C1D8; (**C**) C2D1. (**D**) Principal component analysis demonstrating patient stratification based on clinical outcomes at baseline using the 16-protein signature panel.

WGCNA IDENTIFIED PLASMA PROTEINS CORRELATED WITH PROGRESSION-FREE SURVIVAL (PFS)

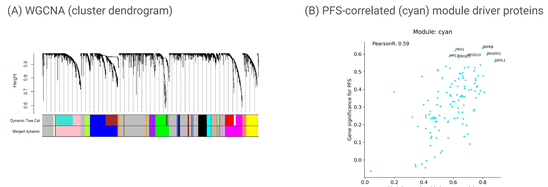

Figure 10 Using weighted gene co-expression network analysis (WGCNA[3]), PFS driver proteins were identified based on module membership and gene significance. (**A**) WGCNA cluster dendrogram; (**B**) PFS-correlated (cyan module) driver proteins.

COX SURVIVAL ANALYSIS

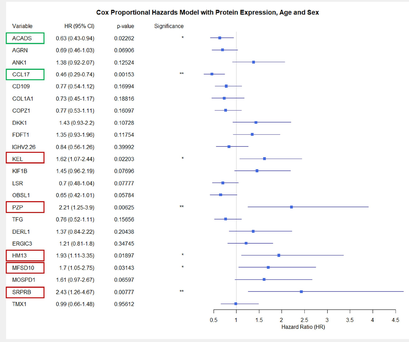

Figure 11 Cox survival analysis. For each protein we fit the multivariate model as Surv(os_time, os) ~ age + sex + protein abundance.

Using ML (**Figure 9**) and WGCNA (**Figure 10**) models we identified 23 predictive biomarkers. Using these biomarkers we performed Cox survival analysis Seven proteins (**Figure 11**) showed significant associations with patient survival: two upregulated in responders (green) and five in non-responders (red), some of which have been previously reported. Notably, CCL17, upregulated in responders, has been linked to enhanced immune cell infiltration and antitumor activity in the LUAD tumor microenvironment[4].

REFERENCES

[1] Gopinath et al., Sci.Rep. 2019

[2] Suomi et al., Plos Comp Bio. 2017

[3] Langfelder and Horvath, BMC Bioinformatics. 2008

[4] Ye et al., Front Cell Dev Biol. 2022

CONCLUSIONS

- **P2 workflow enables unbiased and deep coverage of proteins in the plasma proteome:**
  - **Robustness was demonstrated over 21 plate and 3 independent laboratories**
  - **Linearity is preserved**
  - **P2 works best in EDTA plasma**
- **An integrated pipeline, starting from sample collection, sample analysis and bioinformatics has been established for a phase II NSCLC trial.**
- **A panel of circulating protein signatures has been identified to be closely associated with progression-free survival.**
- **The presented approach provides a powerful platform for identifying predictive biomarkers, enabling more precise patient stratification and potentially improving clinical outcomes.**

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