BIOMARKER DISCOVERY IN NON-SMALL CELL LUNG CANCER ENABLED BY DEEP MULTI-OMICS PROFILING OF PROTEINS, METABOLITES, TRANSCRIPTS, AND CELL-FREE DNA IN BLOOD

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INTRODUCTION

- Lung cancer is a major cause of cancer-related deaths in the United States, with an estimated 238,340 new cases and 127,070 deaths projected in 2023[1]
- Blood-based liquid biopsies hold promise for reducing morbidity and mortality from lung cancer through early detection of disease, identification of patients most likely to benefit from therapy, monitoring of treatment efficacy, and detection of residual disease[2]
- PrognomiQ's multi-omics platform can comprehensively profile proteins, metabolites, lipids, mRNA, and cell-free DNA in blood samples, providing a powerful tool for developing liquid biopsy tests with high sensitivity and specificity for lung cancer
- This platform can offer deep insights into disease biology and has the potential to enable the development of tests with high sensitivity and specificity for the early detection of other cancer types

OBJECTIVE

- To investigate the potential of multi-omics assays to detect and quantify non-small-cell lung cancer (NSCLC) biomarkers in blood samples
- To assess if the biomarkers identified by different assays represent distinct aspects of disease biology

RESULTS

TABLE 1. Number of total and statistically significant features detected for each omics type.

Analyte Type	# Subjects	Total # of Features	# of Significant Features (adj. p-value < 0.05)
Metabolomic	1031	410	232
Lipidomic	1031	605	210
Untargeted Proteomics	990	9,868	2,756
CNV	966	27,143	4,790
RNA transcript	857	109,070	30,242
Fragmentomics	966	329	57

CNV, copy number variation.

FIGURE 1. Biomarkers differentiating between non-cancer and cancer (NSCLC) cohorts spanned the full genotype-to-phenotype spectrum.

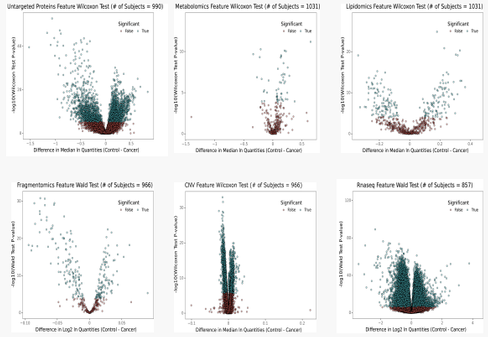
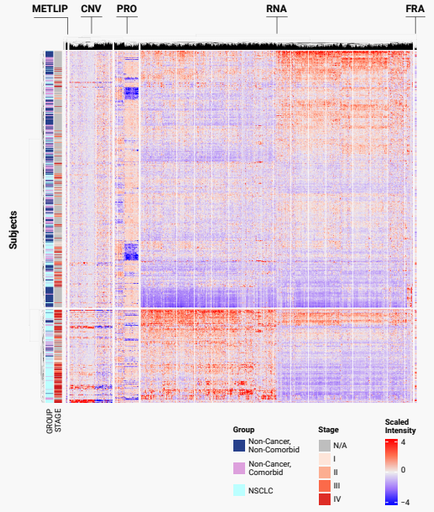


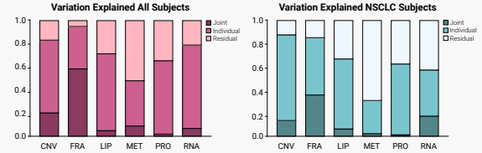
FIGURE 2. Unsupervised clustering on differential biomarkers showed heterogeneity across disease states.



CNV, copy number variation; FRA, fragmentomics; LIP, lipidomics; METLIP, metabolomics and lipidomics; NSCLC, non-small cell lung cancer; PRO, proteomics.

- A substantial number of cancer subjects clustered with non-cancer subjects, even at late-stage
- Supervised machine learning approaches may better separate these groups

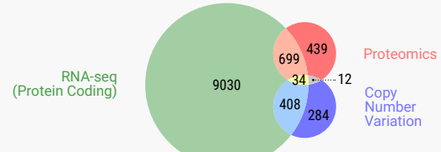
FIGURE 3. Variance decomposition illustrated that shared and unique aspects of biology are captured by each omic type.



CNV, copy number variants; FRA, fragmentomics; LIP, lipidomics; MET, metabolomics; PRO, proteomics.

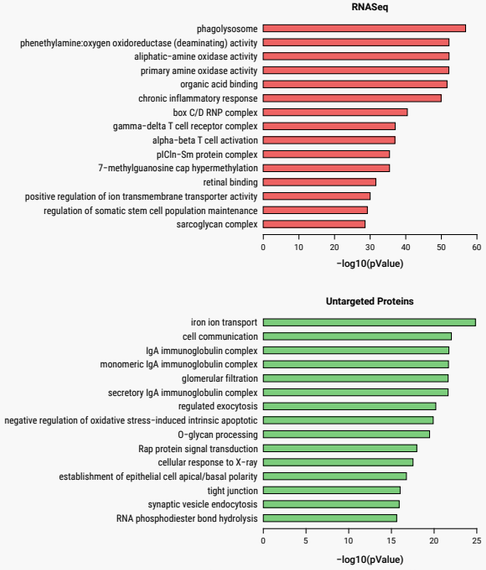
- No single omic type captured all the biological variance
- There was shared biology across the different omics, i.e. the joint component, across which a shared biological signal may be uncovered
- There was also unique biology captured by each individual assay, i.e. the individual component

FIGURE 4. Multi-omics readouts can highlight biomarkers that are shared between omics types.



- Examination of the overlap beteween multi-omics assays can prioritize biomarker candidates
- We focused on differentially expressed plasma proteins for whom the encoding transcript (from RNA-seq) as well as encompassing genomic region (from cell-free DNA) were also differentially abundant
- 34 protein coding genes were identified, including *RPLP0*, *LDHB*, and some KRAS-dependent genes

FIGURE 5. Different molecular assays captured analytes from distinct biological processes.



- Gene set enrichment analysis of mRNA transcripts and proteins differentially expressed between cancer and non-cancer subjects identified multiple gene-ontology terms associated with cancer, including amine-oxidase activity and IgA immunoglobulin complex, respectively

METHODS

Study Design and Sample Collection

- This was a cross-sectional, multi-center, case-control study enrolling 1,031 participants, divided into 3 cohorts: biopsy-confirmed, treatment-naïve NSCLC subjects, non-cancer subjects with comorbidities, and non-cancer subjects without comorbidities
- Blood samples were collected from all subjects and 6 different omics readouts were obtained from plasma: liquid chromatography-mass spectrometry-based proteomics, metabolomics, lipidomics, fragmentomics, copy number variation (CNV), and transcriptomics

Sample and Data Processing

- Individual assay samples were quality controlled, prepared, and processed using field-standard methods for their specific omic type
- Hemolyzed samples were excluded
- Quantitation and normalization were done using field-standard methods specific to each omic type

Data Analyses

- Univariate analyses were performed to identify analyte features differentiating cancer from non-cancer subjects for each assay
- Individual p-values were adjusted for multiple hypotheses testing using the Bonferroni correction to control the family-wise error rate
- Unsupervised clustering was used to investigate if subjects naturally grouped into clusters associated with disease status
- Overlapping and non-overlapping variance analysis was performed with JIVE3 analytical tools
- Gene set enrichment analysis was performed to understand associations with disease biology

CONCLUSIONS

- Multiple biomarkers for NSCLC were detected using blood-based assays across all omics types
- The identified biomarkers demonstrated a broad ability to distinguish between individuals with NSCLC and without cancer

REFERENCES

1. Rolfo C, et al. *J. Thorac. Oncol.* 2021;16(10):1647-1662.
2. Sholl LM, et al. *Arch Pathol Lab Med.* 2016;140(8): 825-829.

DISCLOSURES

Study funded by PrognomiQ, Inc. All employees are current or former employees of PrognomiQ, Inc.

ACKNOWLEDGEMENTS

Funded by PrognomiQ, Inc (San Mateo, CA). Editorial and graphical assistance provided by Prescott Medical Communications Group (Chicago, IL).