Translational Proteomics

LC-MS workflows for diverse omics analysis of plasma samples in a mini cancer cohort using the Orbitrap Astral mass spectrometer

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Abstract

Purpose: To assessed the performance of diverse omics workflow for plasma analysis on the Thermo Scientific™ Orbitrap™ Astral™ mass spectrometer (MS) in a mini cohort of pre-classified diverse cancer and non-cancer samples, enabling larger-scale cancer cohort studies, and population-scale, translational research.

Methods: Proteomic analysis of plasma samples from a mini cancer cohort and age-matched healthy controls was conducted using the Proteograph XT Assay (Seer Inc.). Polar metabolites and lipids were extracted by using a modified Folch method.

The LC-MS analysis for all omics workflow evaluated in the study were all performed on the Orbitrap Astral MS, which was coupled with the Thermo Scientific Vanquish™ Neo UHPLC system.

Results: The Orbitrap Astral MS coupled with Vanquish Neo UHPLC system provide a single platform that enables unprecedented coverage and flexibility of the Orbitrap Astral MS across various omics workflows. Our mini cohort study could pave the way for larger-scale cancer cohort studies employing diverse omics and further advancements in population-scale translational research.

Introduction

The prognosis for cancer patients improves significantly with earlier diagnoses. Current diagnostic methods, such as biopsies and imaging scans, are either invasive or not always easily accessible. In contrast, blood plasma analysis has gained acceptance as a promising approach for biomarker detection. Recent studies have shown that multi-omics analysis enhances accuracy and precision in disease diagnosis due to improved diagnostic features. However, these workflows often require complex instrumentation setups. In our study, we demonstrate that the Orbitrap Astral MS, coupled with the Vanquish Neo UHPLC system, can serve as a single LC-MS platform to perform comprehensive analyses of proteomics, metabolomics, and lipidomics. This effort lays the foundation for diverse omics analysis in translational research.

In the present study, we evaluated the performance of a high-throughput and in-depth workflow for diverse omics analysis using the Orbitrap Astral MS on a small group of pre-classified diverse cancer and non-cancer samples. This initial assessment of the workflows demonstrate that Orbitrap Astral MS together with Vanquish Neo UHPLC provide a single LC-MS platform for streamlined proteomics, metabolomics and lipidomics analysis in translational research.

Materials and methods

Sample preparation

Plasma samples of various cancers, including B-cell lymphoma, colorectal, lung, ovarian and pancreatic cancer together with age- and ethnicity-matched healthy plasma samples were purchased from Discovery Life Sciences and processed from the same site (**Table 1**).

The plasma proteins were enriched by mixing 240 µL of plasma sample with each of the two nanoparticles suspension (NPs) with the Proteograph XT Assay Kit (Seer Inc.) according to manufacturer's instruction (**Figure1**).

Metabolites and Lipids were extracted from 100 µL of plasma using Folch Method. The lipids are partitioned in the upper organic phase of chloroform and the polar metabolites in the lower aqueous phase. After separation, both phases were dried down under nitrogen and resuspended in 100 µL of resuspension solvents composed of 50% IPA/50% ACN for lipids and 50% ACN/50% H_2O for metabolites.

LC-MS methods

For proteomics analysis, plasma samples were loaded onto an IonOpticks Frontier 60x75 C18 UHPLC column. The peptides were loaded onto column with direct injection mode for 16 SPD by using a Thermo Scientific Vanquish Neo UHPLC system. The eluted peptides were analyzed on an Orbitrap Astral MS operated in narrow window DIA mode (**Figure 2**).

For metabolomics and lipidomics analysis, Thermo Scientific Vanquish Horizon UHPLC system was used for high flow separations. HILIC based separation was utilized for the polar metabolites with reversed phase separation for the lipids. The detection was done using the Orbitrap Astral MS in DDA mode (**Table 2**).

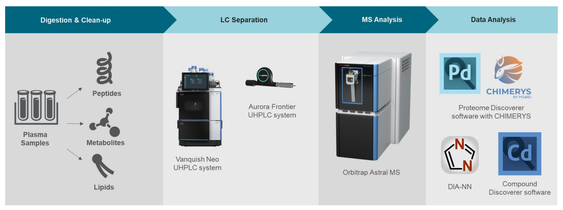
Data analysis

The proteomics data has been processed by DIA-NN (v1.8.1) or Thermo Scientific™ Proteome Discoverer™ with CHIMERYS™ intelligent search algorithm by MSAID. Metabolomics and lipidomics data was analyzed using Compound Discoverer 3.4. The lipidomics data was analyzed within the software utilizing the LipidSearch node.

To integrate the data, results from different workflows were z-scored and combined. UMAP (Uniform Manifold Approximation and Projection) was utilized to visualize analytes with similar variations in proximity.

The resulting tables were imported to Python for downstream data analysis and visualization.

Figure 1. Single platform for diverse omics analysis



Results

Orbitrap Astral mass spectrometer enables high-throughput and maximized coverage proteomics workflows

In-depth proteomic analysis has led to an increased number of features for precise and accurate sample classification. Here, we present a proof-of-concept study showcasing a workflow that offers statistical power for early biomarker discovery. In this mini cohort study, plasma samples from various cancers, including B-cell lymphoma, colorectal, lung, ovarian, and pancreatic cancer, as well as age-, gender-, and ethnicity-matched healthy plasma samples, were prepared and analyzed using an automated, high-throughput approach with the Proteograph XT Assay coupled with an Orbitrap Astral mass spectrometer.

We employed 16 SPD proteomics workflows for in-depth proteome analysis. As a result, we identified over 9,700 protein groups (**Figure 3A**). Furthermore, the quantitation precision was evidenced by a CV of less than 10% (**Figure 3B**). Our data demonstrate the exceptional performance of the Orbitrap Astral MS in plasma proteomics analysis (**Figure 3C**).

Table 1. List of samples included in the mini cancer cohort study.

Plasma Sample	Ethnicity	Gender
Healthy	White	Male
	White	Female
	Black	Female
Colorectal Cancer	Black	Female
	White	Male
Lung Cancer	Black	Female
	White	Male
B Cell Lymphoma	White	Female
	Black	Female
Ovarian Cancer	Black	Female
	White	Female
Pancreatic Cancer	Black	Female
	White	Male

Figure 2. Complete 'end-to-end' LC-MS workflow.

(A)

Time	Duration [min]	Flow [µl/min]	%B	Volume [µl]	No. of Column Volumes
0.000	Run				
0.000	0.000	2.000	10.0	0.00	0.00
0.300	0.300	2.000	10.0	0.60	0.34
0.600	0.300	0.800	10.0	0.42	0.24
13.600	13.000	0.800	22.5	10.40	5.86
20.500	6.900	0.800	35.0	5.52	3.11
20.900	0.400	2.000	55.0	0.56	0.32
20.900	Column Wash				
21.400	0.500	2.000	99.0	1.00	0.56
22.300	0.900	2.000	99.0	1.80	1.01
22.300	Stop Run				
22.300	Column Equilibration				

(B)

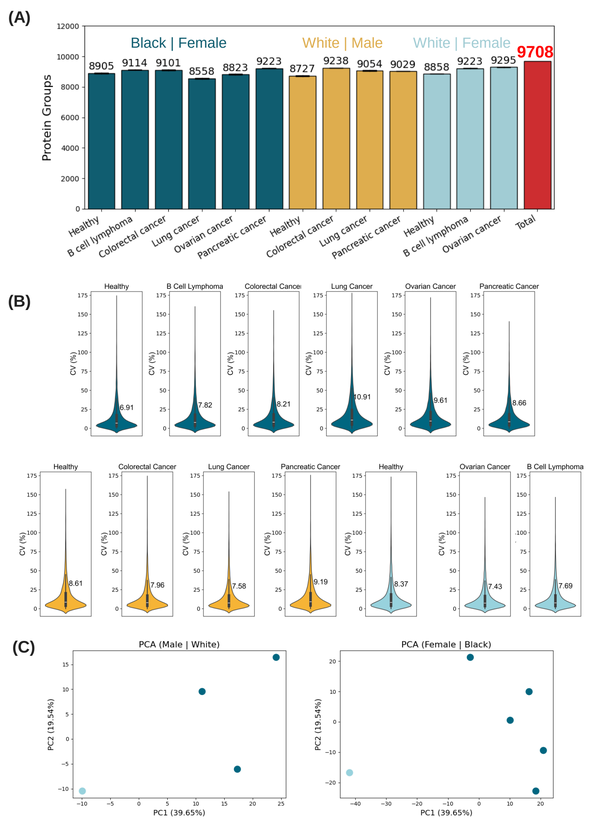
Precursor Mass Range (m/z)	380-980	RF Lens (%)	40
DIA Window Type	Auto	AGC Target	Custom
Isolation Window (m/z)	3	Normalized AGC Target (%)	500
Window Overlap (m/z)	0	Maximum Injection Time (ms)	7
Window Placement Optimization	On	Microscans	1
Number Of Scan Events	200	Data Type	Centroid
DIA Window Mode	m/z Range	Polarity	Positive
Collision Energy Type	Normalized	Source Fragmentation	☐
HCD Collision Energy (%)	25	Loop Control	Time
Detector Type	Astral	Time (sec)	0.6
TMT	Off	Scan Description	
Scan Range (m/z)	150-2000		

(A) Separation column gradient for High-throughput (60 SPD) and maximized coverage (16 SPD) workflow. **(B)** Narrow window DIA parameters on Orbitrap Astral mass spectrometer.

Table 2. Parameters for metabolomics and lipidomics study.

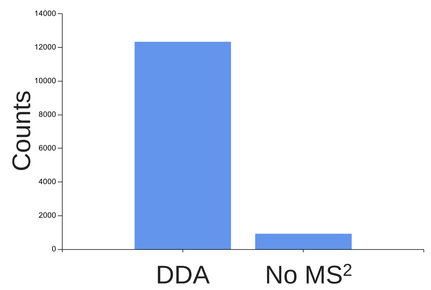
Parameters	Metabolomics	Lipidomics
Analytical Column	Atlantis Premier BEH Z-HILIC (Waters)	Thermo Scientific™ Accucore™ C30
Column Dimensions	1.7 µm, 2.1 mm × 100 mm	2.6 µm, 150 x 2.1 mm
Mobile Phase A	20 mM ammonium carbonate in water with 0.25% (v/v) ammonium hydroxide (pH 9.55)	60:40 ACN:H2O + 0.1% Formic acid + 10 mM Ammonium formate
Mobile Phase B	Acetonitrile	88:10:2 IPA:ACN:H2O + 0.1% Formic acid + 10 mM Ammonium formate
Flow Rate	0.4 mL/min	0.26 mL/min
Gradient Length	30 min	30 min
Spray Voltage (Pos)	4000	4000
Spray Voltage (Neg)	3500	3500
Sheath Gas	40	35
Aux Gas	10	8
Sweep Gas	2	2
RF	50	65
ITT Temperature	325	325
Vapourizer Temperature	350	350
Cycle Time	0.6	0.6

Figure 3. High-throughput and maximized coverage plasma proteomics analysis on the Orbitrap Astral MS.



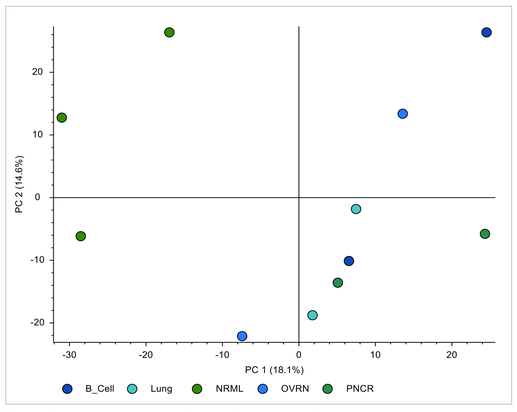
(A) Bar charts showing the number of protein and peptide groups of Seer Proteograph XT enriched plasma from the high-throughput 60 SPD and maximized coverage 16 SPD methods. (B) Coefficient of variance percentage (CV%) to evaluate quantitation precision of the proteomics workflow. (C) Principal component analysis of ethnicity-, gender- and age-matched enriched plasma samples.

Figure 4. Unparallel depth of Orbitrap Astral MS in metabolomics and lipidomics analysis.



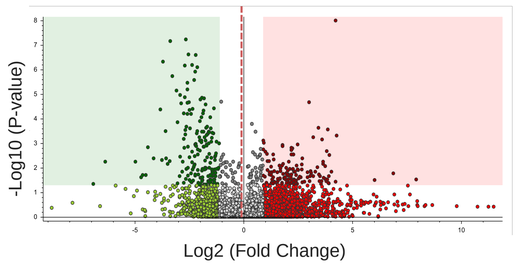
Bar chart demonstrating more than 95% of all features detected in the metabolomic runs to have a MS^2 spectra.

Figure 5. Principle component analysis (PCA) of metabolites extracted from plasma.



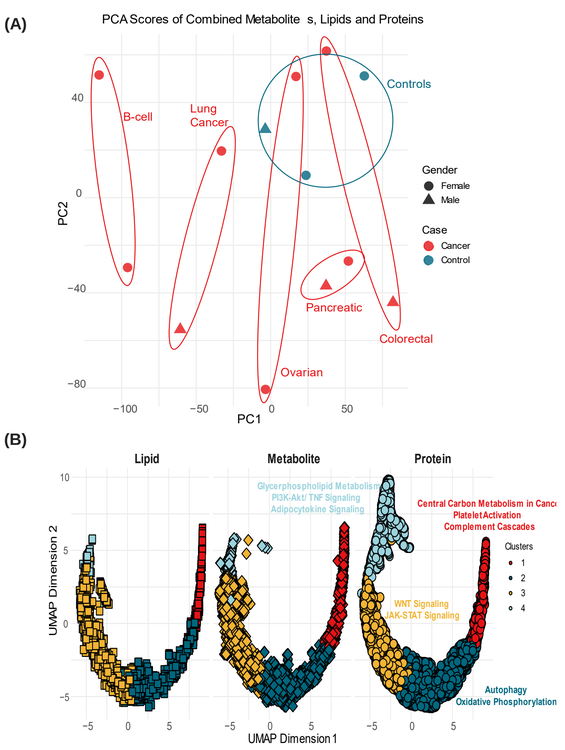
PCA plot showing a segregation of the metabolite from normal plasma and from the various cancer types. The first principal component (PC1) accounts for 18% of the overall variability; the second principal component (PC2) accounts for 14% of the overall variability.

Figure 6. Differential analysis of lipids extracted from healthy plasma vs. B-Cell lymphoma.



Volcano plot showing the differential quantity of 177 Lipids downregulated and 142 lipids upregulated.

Figure 7. Diverse workflows afforded by Orbitrap Astral MS enables insightful biological studies in translational research.



(A) Unsupervised machine learning, principal component analysis (PCA), shows separation, not only between cancer and controls but between different cancer types. (B) Uniform manifold approximation and projection (UMAP) of combined 16,440 analytes faceted between the lipids, metabolilites and proteins illustrates the proximity of features that exhibit similar variation.

Principal component analysis (PCA) reveals distinct molecular variation among samples, enabling the unsupervised separation of cancer types, particularly B-cell, lung, and pancreatic cancers. This differentiation suggests that each cancer type exhibits unique biochemical characteristics that set them apart from both controls and other malignancies.

Complementing this, uniform manifold approximation and projection (UMAP) further delineates the molecular landscape by clustering analytes based on shared features across lipidomic, metabolomic, and proteomic layers. Within the UMAP clusters, distinct pathway-level associations emerge, reflecting the diverse signaling mechanisms driving different cancers. Cluster 1 is enriched with pathways involved in central carbon metabolism of cancer and the complement cascade, highlighting metabolic reprogramming and immune interactions. Cluster 2 is characterized by autophagy-related pathways, suggesting a role in tumor survival and adaptation. Cluster 3 includes pathways linked to WNT and JAK-STAT signaling, both critical regulators of cancer progression and immune evasion. Finally, Cluster 4 is associated with glycerophospholipid metabolism and TNF/AKT signaling, indicating alterations in lipid-mediated signaling and inflammatory responses.

These findings underscore how multi-omic profiling, combined with Orbitrap Atral MS, can uncover cancer-specific metabolic and signaling alterations at unprecedented numbers of annotated features, providing insight into the distinct molecular landscapes that define different cancer types.

Conclusions

- Plasma samples processed with Seer's Proteograph XT Assay and analyzed on an Orbitrap Astral MS identified ~10,000 protein groups using DIA-NN software with CV of ~ 10%.
- Orbitrap Astral MS identified over 15,000 compounds (putative metabolites and lipids) using Compound Discoverer 3.4 and LipidSearch.
- Combined and z-scored data from different workflows, visualized with UMAP, and clustered hierarchically, revealing biological insights and demonstrating the Orbitrap Astral MS's comprehensive omics coverage.

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