



# Metabolic Profiling of Ovarian Cancer Ascites Using high performance liquid chromatography-tandem Ion Mobility-High-Resolution Mass Spectrometry

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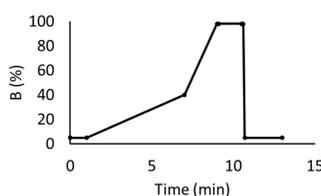
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## Introduction

- **Ovarian cancer (OC)** is the primary cancer most frequently associated with the abnormal production of peritoneal fluid, called **malignant ascites** [1].
- In a previous study by Yang et al. [2], **NMR-based metabolomics** (600 MHz) was applied to identify the differences and correlations between metabolites in ascites from OC patients at different clinical stages.
- **4D-metabolomics approach** was applied to the same samples used for NMR before in order to obtain complementary information. Compared to the traditional 3D-metabolomics LC-MS/MS workflow (retention time, m/z and MS/MS), the integration of **ion mobility** as an additional dimension significantly improves compound identification confidence by providing collisional cross section (**CCS**) values [3].
- samples were analyzed using reversed-phase liquid chromatography (RPLC) and hydrophilic interaction liquid chromatography (HILIC), coupled with **Ion Mobility-High-Resolution Mass Spectrometry (IM-HRMS)** in parallel accumulation-serial fragmentation (PASEF) scan mode on a Bruker timsTOF pro2 system and MetaboScape workstation.

## RPLC Condition

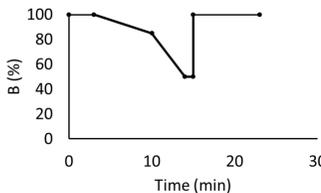
- Mobile phase A: : 0.1% formic acid in water
- Mobile phase B: 0.1% formic acid in acetonitrile



Column	Intensity Solo C18 2x100 mm, 2.0 μm
Col. Comp.	50°C
Flow Rate	0.40 mL/min

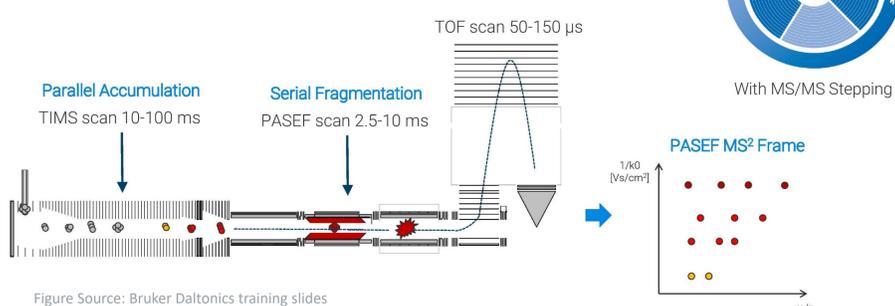
## HILIC Condition

- Mobile phase A: : 0.1% formic acid in water
- Mobile phase B: 10 mM NH<sub>4</sub>HCO<sub>2</sub> and 0.1% formic acid in acetonitrile



Column	ACQUITY UPLC BEH Amid Column, 2.1x150 mm, 1.7 μm
Col. Comp.	40°C
Flow Rate	0.50 mL/min

## PASEF Scan Mode with Stepping strategy



- Multiple ions are accumulated simultaneously in the ion funnel and sequentially transferred and fragmented.
- TIMS Stepping: Multiple TIMS ramps are measured and merged together in a single final acquisition.
- MS/MS Stepping: Masses transferred in each step can be fragmented with two customizable collision energies (CEs).

## Conclusions

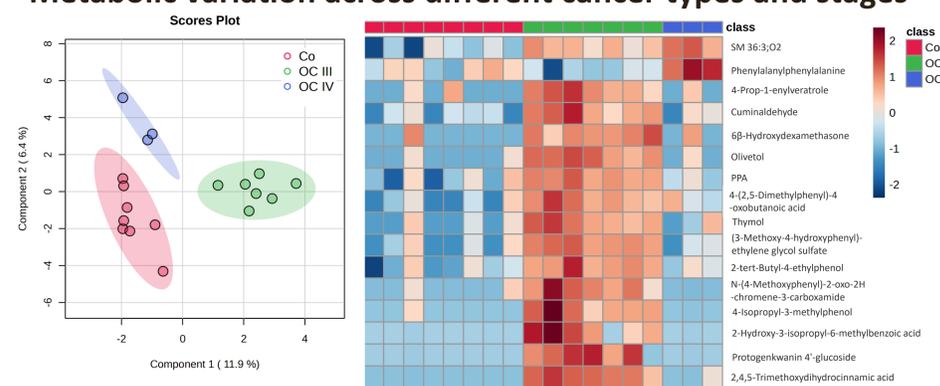
- Over 700 metabolites and lipids were annotated with high confidence using 4D annotation.
- 34 metabolites were unique to NMR, while 564 metabolites were unique to LC-MS, showing the high complementarity of both techniques.
- Metabolomic differences were successfully identified, distinguishing clinical stages of ovarian cancer as well as varying histological types.

## Ascites metabolite coverage with LC-MS and NMR



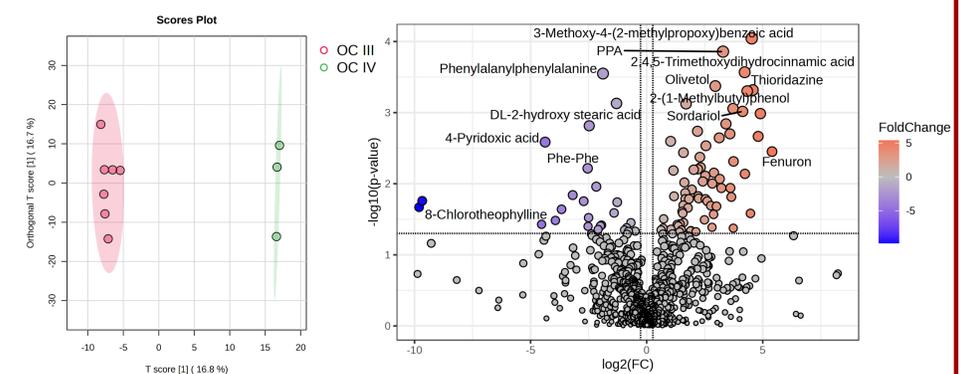
- A Venn diagram showing the overlap of metabolites between two studies using HPLC-IM-HRMS and NMR. Only eleven metabolites in total are congruent between the two platforms.

## Metabolic variation across different cancer types and stages



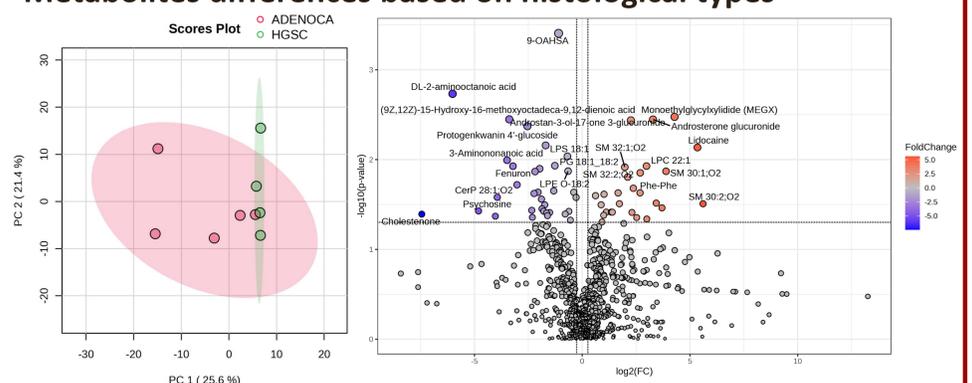
- Sparse Partial Least Squares Discriminant Analysis (sPLS-DA) scores plot comparing ovarian cancer at clinical stages III, IV, and a control group from other cancer types.
- A heatmap of metabolites in ovarian cancer at clinical stages III, IV, and the control. Out of 757 annotated metabolites, 16 significant metabolites ( $p < 0.05$ ) were identified.

## Metabolomics profiling on different stages of ovarian cancer



- Orthogonal Partial least squares discriminant analysis (oPLS-DA) scores plot from OC at clinical stage III and IV.
- Volcano plot of differential expression results of OC at clinical stage III and IV. 96 significant metabolites ( $p < 0.05$ ) were found out of 760 annotated metabolites.

## Metabolites differences based on histological types



- Principal Component Analysis (PCA) scores plot and volcano plot for ovarian cancer, specifically comparing Adenocarcinoma (subtype unknown) and High-Grade Serous Carcinoma (HGSC).
- Out of 755 annotated metabolites, 61 significant metabolites ( $p < 0.05$ ) were identified.

## Acknowledgements

The authors would like to thank Gyuntae Bae for support with data analysis and Bruker Daltonics for the cooperation and their continuous support.



## References

- (1) Kipps E, et al., *Nat Rev Cancer*. 2013;13(4):273–82.
- (2) Yang et al., *Journal of Translational Medicine* 2022; 20:581.
- (3) Pieter V, et al., *Analytical Chemistry* 2018;90 (19):11643-11650.