

Book of Abstracts

Conference Program

Time	Activity
09:00–09:20	Participants Registration
09:20–09:25	Opening Remarks and Institutional Welcome
09:25–10:10	Plenary Lecture: ***Giovanni Boniolo:*** *"Science: What else"*
10:10-10:45	**Coffee Break** — IFOM Cafeteria
10:45–11:35	**Oral Session I** — *Conference Hall*
	Mobin Pourvali: *Identification of small non-canonical proteins in epithelioid Sarcoma by SP3 enrichment and N-terminomics*
	Paola Fulghieri: *Integrated EPI- and proteomic profiling improves prediction of recurrence in low-grade meningiomas*
	Alice Di Bernardo: *Exploring extracellular vesicles by Mass Spectrometry: from molecular profiling to therapeutic platforms*
11:35-12:20	Invited speaker: ***Joelle Vinh:*** *Proteomic study of caprine dairy exploitation on the Iranian Plateau from the seventh millennium BC*
12:20–13:45	**Poster Session** — Meeting Hall 1+2
13:45–14:45	**Lunch** — IFOM Cafeteria
14:45–15:15	**Oral Session II** — *Conference Hall*
	Nicole Croci: *LC-HRMS lipidomics supports targeting ceramide glycosylation to delay resistance to Osimertinib in EGFR-mutated Non-Small Cell Lung Cancer*
	Nicoletta Lionetti: *Novel insight from p-cresyl and indoxyl sulfate metabolomic analysis in preclinical models of acute kidney injury*
15:15–17:00	**Workshop:** "Career in MS" — *Conference Hall*
17:00–17:30	Final Discussion and Closing Remarks

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FISH EMBRYO TOXICITY TESTING COMBINED WITH UNTARGETED LIPIDOMICS REVEALS LIPIDOMIC ALTERATIONS FOLLOWING SULFAMETHOXAZOLE AND TRIMETHOPRIM EXPOSURE

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Antibiotics released into aquatic environments may affect non-target organisms by disrupting lipid metabolism [1]. Untargeted lipidomics can reveal molecular responses to environmental stressors in zebrafish embryos, complementing conventional toxicity endpoints [2]. This study combined fish embryo toxicity testing, behavioural assessment and untargeted lipidomics to investigate the effects of sulfamethoxazole (SMX) and trimethoprim (TRIM) in zebrafish (Danio rerio). Preliminary exposure assays were performed into 120 hours post-fertilization, followed by locomotor assessment. A separate exposure experiment was conducted for lipidomic profiling, comparing control larvae with larvae exposed to SMX (5 mg/L), TRIM (1 mg/L), or their combination at the same individual concentrations. Lipids extracted from pooled larvae were analysed by HPLC-HRMS, using a Orbitrap Q-Exactive in positive and negative electrospray ionization modes. Untargeted profiling annotated 436 lipid species. Principal component analysis of polar lipids explained 65.4% of the variance in the first two components, and pairwise PERMANOVA detected significant differences between each exposure group and the control. Combined exposure also differed from both individual treatments. These findings suggest untargeted lipidomics may reveal antibiotic-associated molecular responses during zebrafish early development.

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POSTER

LINKING KINASE SIGNALING TO PRMT1 REGULATION IN OVARIAN CANCER CHEMORESISTANCE

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Epithelial ovarian cancer (EOC) responds well to initial chemotherapy but relapse, associated with chemoresistance, leads to poor survival outcome. Protein arginine methyltransferases (PRMTs), the enzymes that monomethylate or dimethylate the guanidino group of arginine, have been linked to the development of chemoresistance in various cancers. Our group previously showed that upon cisplatin-treatment, PRMT1 undergoes phosphorylation-dependent translocation to chromatin, where it dimethylates arginine 3 of histone H4 consequently enhancing the transcription of prosurvival genes in chemoresistant EOC cells.

The aim of this project is to elucidate the signaling cascade governing PRMT1 phosphorylation in response to CDDP. Specifically, we seek to identify and quantify CDDP-responsive phosphorylation sites on PRMT1, determine the responsible kinase(s), and assess how PRMT1 phosphorylation modulates substrate affinity, protein interactions, and subcellular localization, converging phosphorylation signaling cascades induced by cisplatin that shift the arginine(R) methyl-proteome.

To this purpose, we conducted a comprehensive proteomic and phosphoproteomic analysis of chemoresistant EOC cells challenged with cisplatin and kinase inhibitors. Utilizing two sequential phosphoenrichment steps using Ti-IMAC beads, we identified over 20000 phosphosites from 7000 proteins across all conditions on the Orbitrap Astral. Interestingly, among these data, we detected four distinct phosphorylation sites on PRMT1, providing initial insight into its potential regulation and setting the stage for further functional validation. Integration of total proteome and phosphoproteome data enabled inference of differential kinase activities and mapping of altered signaling pathways. This large-scale approach revealed distinct kinase–substrate dependencies associated with cisplatin-induced stress responses in chemoresistant EOC.

Acknowledgements
AIRC foundation

ORAL COMMUNICATION

LC-HRMS LIPIDOMICS SUPPORTS TARGETING CERAMIDE GLYCOSYLATION TO DELAY RESISTANCE TO OSIMERTINIB IN EGFR-MUTATED NON-SMALL CELL LUNG CANCER

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Non-small-cell lung cancer (NSCLC) is a major global health burden, with epidermal growth factor receptor (EGFR) alterations serving as important oncogenic drivers [1]. Resistance to third-generation tyrosine kinase inhibitors, such as osimertinib, remains a major clinical challenge [2]. Among the mechanisms associated with drug resistance, lipid reprogramming has emerged as an important contributor, with glycosphingolipids (GSL) implicated in tumour progression and therapeutic resistance [3]. Here, we used LC-HRMS lipidomic profiling to investigate the relationship between GSL metabolism and osimertinib resistance in EGFR-mutated NSCLC cells and evaluated the effects of glucosylceramide synthase (GCS) inhibition.

NSCLC cells exposed to moderate concentrations of osimertinib developed resistance through the emergence of drug-tolerant persister colonies and showed significant increases in globotriaosylceramides (Gb3). GCS inhibitors reduced Gb3 and its precursors, glucosylceramide (GlcCer) and lactosylceramide (LacCer), in a dose-dependent manner, paralleling the reduction in resistant colony formation. Combining osimertinib with the GCS inhibitor venglustat significantly reduced long-term tumour regrowth in a mouse xenograft model. To investigate the clinical relevance of ceramide glycosylation, we analysed plasma samples from patients with EGFR-mutated NSCLC. LC-MS quantification using the standard-addition method revealed an increase in Gb3-to-ceramide glycosylation ratio for palmitoyl species at disease progression compared with baseline.

As glycosphingolipidomics gains prominence in the development of novel anticancer strategies, these findings highlight the value of LC-HRMS profiling in characterizing GSL alterations associated with osimertinib resistance and support GCS targeting to delay its emergence in EGFR-mutated NSCLC.

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ORAL COMMUNICATION

EXPLORING EXTRACELLULAR VESICLES BY MASS SPECTROMETRY: FROM MOLECULAR PROFILING TO THERAPEUTIC PLATFORMS

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Extracellular vesicles (EVs) are emerging as key mediators of intercellular communication and as promising platforms for therapeutic applications [1]. Their complex molecular composition, reflecting both the cell of origin and its physiological or pathological state, makes EVs particularly attractive targets for mass spectrometry (MS)-based investigation. In this context, the combination of untargeted and targeted MS approaches can provide complementary information, ranging from global molecular profiling to the quantitative determination of selected molecules of interest.

Here, we present MS-based strategies applied to EVs characterization in two complementary biomedical contexts. In the first one, EVs released by drug-sensitive and multidrug-resistant non-small cell lung cancer (NSCLC) cells are investigated to explore molecular alterations associated with drug resistance and with changes in the tumor microenvironment. Untargeted lipidomic and metabolomic profiling was used to characterize EV molecular signatures and to investigate how cellular phenotype and environmental conditions influence their cargo. This approach provides a molecular perspective on EVs as potential mediators of metabolic adaptations associated with multidrug resistance. [2]

In parallel, MS was applied to the characterization of EVs as therapeutic platforms. Targeted LC-MS/MS methods enabled the quantitative analysis of selected compounds, including cholesterol and tacrolimus as a model pharmacologically active cargo, supporting the evaluation of EV composition and drug loading. These targeted analyses provided quantitative information relevant to the development and characterization of engineered EV-based delivery systems.

Together, these applications show the versatility of mass spectrometry for EV research. By integrating untargeted molecular profiling with targeted quantitative analysis, MS can contribute both to understanding the biological information carried by EVs and to evaluating their potential as therapeutic delivery platforms.

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POSTER

METABOLOMIC AND LIPIDOMIC PROFILING OF AMNIOTIC FLUID AT PRETERM BIRTH RELATED TO BRONCHOPULMONARY DYSPLASIA

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Bronchopulmonary dysplasia (BPD) represents one of the most devastating morbidities following preterm birth [1,2]. While the effects of postnatal oxygen toxicity and mechanical ventilation are well understood, prenatal biological variations contributing to BPD are poorly described [3].

Metabolomic and lipidomic profiles of maternal amniotic fluid allow the detection of prenatal alterations related to BPD development. Untargeted analyses were conducted through mass spectrometry coupled with ultra-performance liquid chromatography. Metabolites and lipids were annotated by comparing the accurate mass, retention time, collision cross section, and fragmentation spectra with our in-house laboratory database.

The integration of the two omics approaches revealed distinct profiles capable of identifying preterm newborns at risk of developing BPD, highlighting dysregulation of lipid metabolism and endogenous steroids (Figure 1).

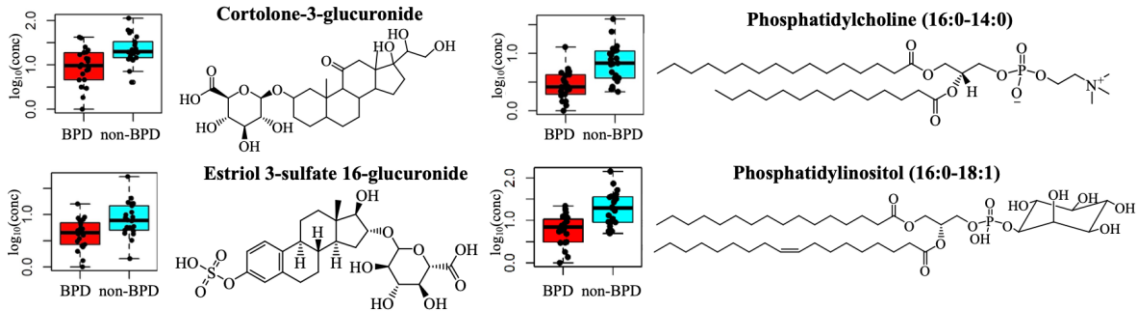


Fig. 1: Boxplots and molecular structures of selected relevant metabolites (left) and lipids (right) in amniotic fluid for BPD (red) and non-BPD (cyan) patients.

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ORAL COMMUNICATION

INTEGRATED EPI- AND PROTEOMIC PROFILING IMPROVES PREDICTION OF RECURRENCE IN LOW-GRADE MENINGIOMAS

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Epigenetic alterations are now well-documented cancer hallmarks. In particular, alterations in the levels of histone post-translational modifications (PTMs) can result in dysregulated protein expression and sustain cancer development and progression [1]. Meningiomas are the most common brain tumours, yet their grading system is often unable to predict the recurrence or the aggressive behaviour of low-grade malignancies, which represent the majority of initial diagnoses [2]. Hence, to unveil potential predictors of meningioma relapse, we not only profiled the global proteome of 162 meningiomas (grade I: n=107; grade II: n=50; grade III: n=5) exploiting the high sensitivity of Orbitrap Astral mass spectrometer, but also characterised the best annotated histone PTMs using a robust, in-house-developed mass spectrometry (MS)-based epi-proteomic workflow. Our combined approach led to the quantification of up to 9,237 proteins and up to 56 histone lysine methylation and acetylation marks. By integrating proteomic and histone PTMs data, we outlined 3 tumour clusters with different molecular signatures and progression-free survival (PFS). Notably, 20% of Grade I clustered among recurrence-prone meningiomas. These tumours were enriched in RNA splicing and chromatin components and showed lower histone H3K27me3 and H4K20me1, alongside increased H3K9me3, uncovering a potential proteomic and epigenetic signature for faster recurrence. While under further verification in an independent cohort of Grade I tissue and liquid biopsies, our data already highlight the potential of MS to enhance the molecular characterisation of meningiomas and to disclose possible targets for future testing of protein modulators and epigenetic drugs.

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Acknowledgements

PRIN2022 (CUPG53D23001530006)MIND: Meningioma epIgeNetic liquiD biopsy;
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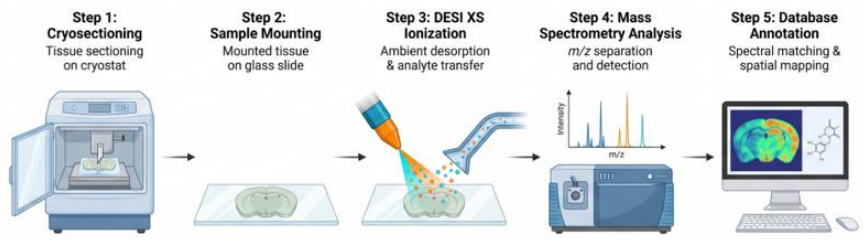
POSTER

AN OPTIMIZED DESI-MS IMAGING WORKFLOW FOR SPATIAL LIPIDOMICS AND METABOLOMICS IN BIOLOGICAL SYSTEMS

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Desorption Electrospray Ionization Mass Spectrometry (DESI-MS) imaging is a powerful ambient ionization technique that enables the spatial distribution analysis of endogenous metabolites and lipids directly from biological samples with minimal preparation [1]. However, its application to complex systems such as 3D in vitro models and native tissues requires rigorous and reproducible optimization. Here, we developed and systematically optimized a DESI-MS imaging workflow on a Synapt XS system (Fig. 1), tuning solvent composition and flow rate, spray voltage, capillary settings, sprayer geometry and scan speed to maximize signal intensity and stability, minimize ion suppression and preserve spatial resolution. The optimized method uses 98% methanol with 0.01% formic acid (with internal Leu-Enk lock mass) at 0.3 L/min, with pixel sizes from 25 to 100 m in positive and negative ion modes. Molecular species were annotated by accurate mass and collision cross section (CCS), with ion mobility fragmentation for selected species. Data processing and multivariate statistical analyses were custom developed in R. The workflow was applied to 3D neuromuscular organoids (in vitro) and murine brain tissue (ex vivo), enabling the spatially resolved detection of lipid species such as PC, PG, PI, TG, PE and PS. This opens the way to relating their spatial distribution to the biology of complex tissues. Overall, by combining robust mass spectrometry workflows with dedicated statistical data treatment, this approach provides a reliable platform for spatial molecular profiling, with potential applications in tissue characterization, drug distribution studies and the assessment of treatment response [2].



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POSTER

HRMS-BASED CHEMICAL CHARACTERIZATION OF NICOTINE POUCHES ON THE ITALIAN MARKET

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Nicotine pouches are an expanding category of tobacco-free oral nicotine products characterized by substantial variability in formulation and nicotine strength, as confirmed by independent studies, which have also reported inconsistencies in product labelling [1,3] and the presence of numerous flavouring agents, additives and other chemical constituents [2]. This variability, together with the potential for substantial nicotine exposure, raises public health concerns and highlights the need for further analytical characterization. However, comprehensive data on products currently available on the Italian market remain limited.

The present study aims to characterize nicotine pouches available on the Italian market by quantifying nicotine content, comparing measured and manufacturer-declared values, and profiling additional constituents. The analytical strategy combines targeted nicotine quantification and untargeted chemical profiling using complementary GC-MS and LC-HRMS approaches.

Preliminary untargeted GC-MS and LC-MS analyses of nicotine-containing products revealed distinct chemical profiles. Nicotine pouch samples showed differences in the occurrence of nicotine-related compounds, flavouring substances and humectant/sweetening agents, highlighting the value of complementary analytical approaches for broader chemical characterization.

The combined targeted and untargeted approach is designed to provide a comprehensive chemical characterization of nicotine pouches marketed in Italy, with particular focus on labelling accuracy and the identification of constituents beyond predefined targets.

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Acknowledgements

This work was supported by Journalism and Science Alliance (JSA) through the project NoPouch: Preventing the Expansion of Nicotine Pouches in Italy.

ORAL COMMUNICATION

NOVEL INSIGHT FROM p-CRESYL AND INDOXYL SULFATE METABOLOMIC ANALYSIS IN PRECLINICAL MODELS OF ACUTE KIDNEY INJURY

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Introduction. p-Cresyl sulfate (pCS) and indoxyl sulfate (IS) are the main protein-bound uremic retention solutes, generated by bacterial catabolism of tyrosine and phenylalanine and of tryptophan respectively [1]. Their accumulation belongs to a gut-kidney axis in which renal impairment and intestinal barrier dysfunction reinforce each other. Here we measured pCS and IS by LC-MS/MS in rat models of renal injury and microbiota manipulation.

Methods. A targeted LC-MS/MS method quantified total serum pCS and IS in a single 6-minute run. After protein precipitation, serum was analysed on a Shimadzu Nexera UPLC coupled to an LCMS8060 CL triple quadrupole in negative ESI-MRM mode and data analysis was performed with LabSolutions. Four rat groups were compared: i) wild type (WT), ii) adenine, iii) adenine plus antibiotic (ApA) and iv) fecal microbiota transplantation (FMT) (Figure 1).

Results and Discussion. Both pCS and IS were significantly increased compared to WT in the ApA group and after FMT ($p<0.05$), with the highest levels observed in the ApA group, and FMT failed to normalize them. pCS and IS are eliminated via tubular secretion, a pathway compromised by adenine-induced tubulointerstitial injury. Once secretory capacity is lost, microbial production is presumably no longer rate-limiting, and restoring the microbiota cannot compensate for a transport defect. Our results position pCS and IS as promising markers of tubular secretory function, highlighting their potential as indicators of renal function complementary to those currently used in clinical practice.

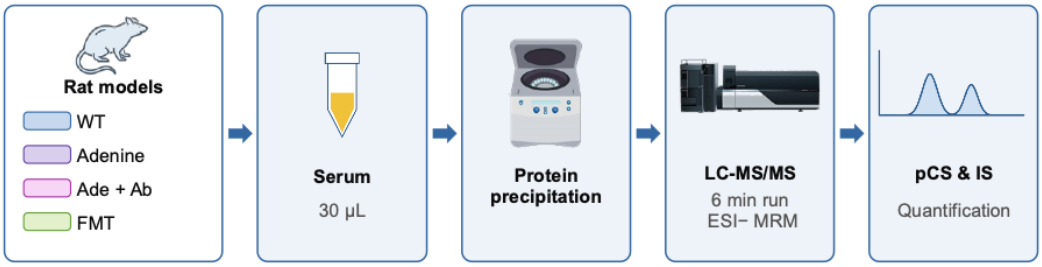


Figure 1. Study workflow

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POSTER

ABAXIAL/ADAXIAL LEAF PROFILING REVEALS EARLY METABOLIC MARKERS OF FLAVESCENCE DORÉE

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Flavescence dorée (FD) is a quarantine grapevine phytoplasma disease transmitted by leafhoppers and represents a serious threat to viticulture, causing substantial yield losses across Europe. FD infection profoundly alters host metabolism, making the identification of disease-associated metabolites crucial for early diagnosis and effective disease management. However, early detection remains challenging because metabolic alterations may occur before the onset of visible symptoms [1]. Moreover, conventional bulk analyses can mask spatially resolved differences between the adaxial and abaxial leaf surfaces, which have distinct physiological functions. Therefore, characterizing surface-specific metabolic responses may provide biomarkers for early FD detection. Here, we used high-resolution untargeted MALDI-MSI to characterize surface-specific metabolic alterations in FD-infected Vitis vinifera cv. Barbera leaves and identify potential biomarkers of pre-symptomatic FD infection. Grafted cuttings were exposed to FD-infected insect vectors, and leaves were sampled at symptom onset. Non-exposed controls, exposed asymptomatic, and exposed symptomatic plants were analyzed separately for adaxial and abaxial leaf surfaces. Samples were coated with CHCA and acquired using AP-MALDI coupled to an Orbitrap Q-Exactive mass spectrometer. Comparing adaxial and abaxial leaf surfaces of the three conditions, an adaxial/abaxial intensity ratio strategy was introduced, amplifying surface-specific metabolic asymmetry and resolving pre-symptomatic changes undetectable by absolute intensities alone. Among 5,100 detected features, 75 were statistically significant using this ratio-based approach, and LC-MS/MS together with database matching putatively annotated 16 candidate biomarkers spanning classes related to primary metabolic reprogramming and inducible defense pathways. These results demonstrate that spatially resolved, surface-paired metabolomics can uncover early diagnostic biomarkers, supporting pre-symptomatic FD detection strategies. The next step is to validate the ratio approach under field conditions.

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POSTER

IMPACT OF MASS SPECTROMETRY ACQUISITION PARAMETERS ON PLASMA PROTEOME COVERAGE BY nanoHPLC-HRMS

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Plasma proteomics represents a powerful approach for investigating physiological and pathological processes through the comprehensive characterization of circulating proteins. The combination of nano-high-performance liquid chromatography with high-resolution mass spectrometry (nanoHPLC-HRMS) provides high sensitivity and analytical performance for protein identification [1]. However, plasma analysis remains particularly challenging because of the extremely wide dynamic range of protein concentrations, with highly abundant proteins potentially masking lower-abundance species.

In this study, different sample preparation and mass spectrometry acquisition strategies were evaluated to improve plasma proteome coverage. Two sample preparation workflows were initially compared: a methanol precipitation-based protocol followed by conventional denaturation, reduction, alkylation and enzymatic digestion, and a urea-based protocol involving protein denaturation, reduction, alkylation and in-solution digestion. The urea-based workflow, followed by peptide purification, was selected for subsequent optimization of data-dependent acquisition parameters.

Particular attention was focused on dynamic exclusion and ddMS_2 analyzer selection. Dynamic exclusion times of 10, 20 and 30 s were systematically evaluated, together with the use of either Orbitrap (OT) or Ion Trap (IT) detection for MS_2 acquisition.

Proteomic data were processed using Proteome Discoverer. At a fixed dynamic exclusion of 30 s, switching MS_2 detection from OT to IT markedly increased proteome depth, from 53 to 97 master protein groups.

These findings demonstrate the strong influence of acquisition settings on plasma proteome coverage and highlight the importance of optimizing both dynamic exclusion and MS_2 detection strategy in nanoHPLC-HRMS-based proteomic workflows.

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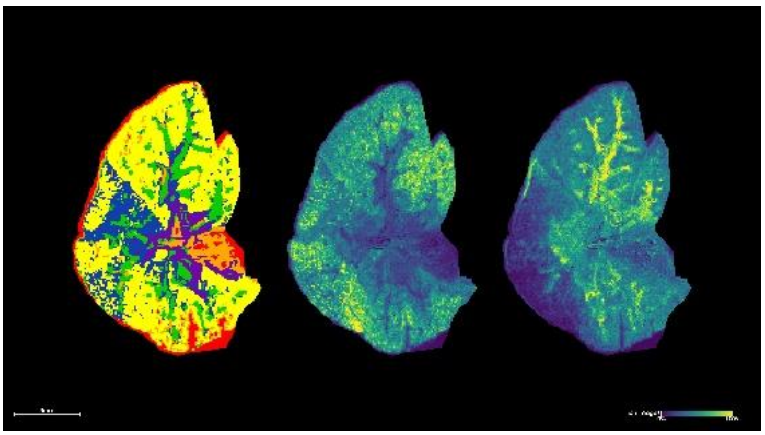
POSTER

EXPLORING SPATIAL LIPID ALTERATIONS IN MURINE LUNG DURING LEGIONELLA PNEUMOPHILA INFECTION USING MALDI-TOF MSI

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Pulmonary lipids play crucial roles in immunity and cellular protection, serving as structural components of pulmonary surfactant. However, their spatial distribution in lung tissue during infection remains poorly understood [1], [2]. *Legionella pneumophila*, a gram-negative bacterium causing severe pneumonia, manipulates host lipid metabolism to facilitate intracellular replication and alter pulmonary homeostasis [3]. Understanding how infection affects the spatial organization of the pulmonary lipidome across distinct lung regions may provide new insights into host-pathogen interactions and tissue-specific responses. MALDI-MSI imaging is a powerful approach to map spatially resolved lipids directly in tissue sections, enabling the investigation of regional heterogeneity that bulk analyses cannot capture. This study aims to develop a data elaboration pipeline for MALDI-TOF MSI to investigate the spatial distribution of pulmonary lipidome in mice infected with different strains of *Legionella pneumophila* and identify infection-associated lipid alterations. Lung samples from non-infected mice and mice infected with wild-type (130b Wadsworth; Corby) and mutant (16D12; TF3/1) strains – where the LPS structure was modified by the mutations- were analysed in positive and negative ion modes using a Bruker ultrafleXtreme ToF/ToF instrument. Lipid classes including PCs, PGs, PSs, and PEs were analysed in SCiLS Lab using class-specific internal standards (PC 12:0, PG 12:0, PE 12:0, PS 14:0). Preliminary MALDI-MSI images in negative ion-mode confirmed the capability of our pipeline to identify discriminant lipids distinguishing airways from parenchyma through segmentation and spatial co-localization analysis in non-infected mouse lungs. The lipid signatures identified were consistent with literature data, validating our analytical approach and providing the foundation for pipeline application to infected samples to identify infection-associated lipid alterations.



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ORAL COMMUNICATION, POSTER

IDENTIFICATION OF SMALL NON-CANONICAL PROTEINS IN EPITHELIOID SARCOMA BY SP3 ENRICHMENT AND N-TERMINOMICS

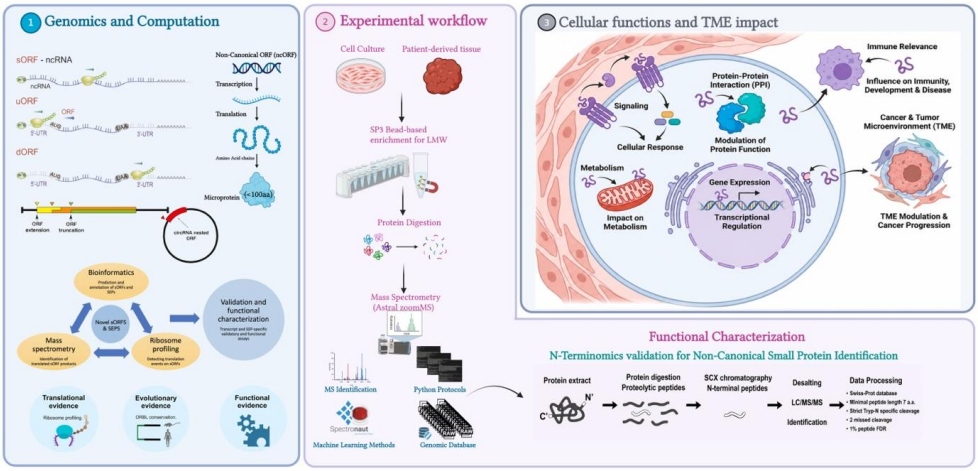
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For decades, the human genome was defined by nearly 20,000 protein coding genes, and anything shorter than 100 codons was dismissed as statistical noise. That picture is now changing. Recent evidence shows that microproteins encoded by small open reading frames carry fundamental functions in cancer biology; however, they remain difficult to detect with current proteomics methods. Our study integrates computational prediction, proteomics and orthogonal validation to uncover this hidden proteome in epithelioid sarcoma. To overcome microprotein loss during sample preparation, we used an optimised single-pot, solid-phase-enhanced sample preparation workflow based on carboxylate-coated magnetic beads. By tuning the organic solvent concentration, we adjusted the binding conditions to retention of small, low-molecular-weight species on the beads. Enriched proteins were digested and analysed on an ultrasensitive Orbitrap Astral Zoom mass spectrometer. Identification combined machine learning based scoring in Spectronaut with a dual repository search of OpenProt and SmProt alongside the canonical reference proteome. To increase identification stringency, N-terminomics was incorporated as an independent validation layer, in which protein N-termini were selectively enriched by strong cation exchange chromatography after digestion. Because each translated protein carries a unique N-terminus, recovery of the peptide at the very start of a non-canonical open reading frame supports the annotated initiation site and provides orthogonal evidence that the microprotein is produced.

We identified nearly 4,000 microproteins absent from canonical reference databases. The start codon distribution derived from the FASTA headers shows that close to seven in ten of them initiate at a codon other than AUG, such as GUG or CUG, while N-terminomics experimentally confirmed 386 initiation sites, 301 of which agreed with the annotated start codon. Among the quantifiable microproteins, 135 were differentially abundant between tumour and healthy cells (89 up, 46 down), and 11 remained significant after Benjamini Hochberg correction for multiple testing; some of these behaved in the opposite direction to their own host gene, indicating regulation independent of the canonical transcript. Overall, the optimised workflow recovered a small proteome in a rare sarcoma and it leaves a prioritised list of ten candidates for targeted validation and functional characterisation.

Graphical abstract



Overview of the study, from non-canonical open reading frame (ncORF) prediction to functional characterisation. (1) Origins of ncORFs – small ORFs (sORFs) in non-coding RNAs, upstream ORFs (uORFs), downstream ORFs (dORFs), extended ORFs and circRNA-nested ORFs – and how bioinformatics, ribosome profiling and mass spectrometry are combined with translational, evolutionary and functional evidence before a microprotein is accepted as genuine. (2) Workflow of this study: cultured cells and patient-derived tissue undergo SP3 bead-based enrichment of low-molecular-weight proteins, digestion and analysis on an Orbitrap Astral Zoom mass spectrometer; identifications are obtained with machine-learning-based scoring in Spectronaut, custom Python scripts and a custom genomic database. (3) Cellular roles of microproteins, including signalling, metabolism, gene expression, protein–protein interactions, immunity and the tumour microenvironment. (4) the N-terminomics validation step, in which N-terminal peptides are enriched by SCX chromatography after digestion and searched under strict Tryp-N rules at 1% peptide false discovery rate (FDR). Created with BioRender.com.

Acknowledgements

This work was carried out at Fondazione IFOM, AIRC Institute of Molecular Oncology, Milan and Cogentech SB Srl Proteomics & Metabolomics Core Facility.

POSTER

GC-MS VS LC-MS/MS FOR FATTY ACID ANALYSIS IN A MUCIN-BASED 3D GUT MODEL

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Short-chain fatty acids (SCFAs), mainly acetic (AA), propionic (PA) and butyric acid (BA), are key markers of gut microbiota activity. Gut3Gel, a mucin-based hydrogel, reproduces the intestinal mucus barrier more closely than conventional cultures, but its viscous matrix makes fatty acid analysis challenging. This study compares two derivatization-based mass spectrometry methods, GC-MS and LC-MS/MS, for the analysis of fatty acids in Gut3Gel and in Brain Heart Infusion (BHI) medium. GC-MS involved silylation with N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) [1], whereas LC-MS/MS involved EDC-mediated coupling with 4-bromo-N-methylbenzylamine (4-BNMA) [2,3]. Both methods were calibrated in matrix, using propionic acid-d5 as internal standard. GC-MS showed good linearity ($R^2 > 0.998$), precision (RSD 6.7–10.7%) and LOQs of 0.1–1.6 µg/mL for AA, PA and BA. LC-MS/MS showed lower precision (RSD 12.4–14.4%) and a narrower working range for PA and BA, and had three main limitations: acetic acid in the blanks exceeded the highest standard, recovery after extraction was low, and the matrix effect was variable. GC-MS was therefore used to follow the fatty acid production of fecal microbiota from healthy donors over 72 h in BHI (aerobic and anaerobic conditions) and in Gut3Gel (oxygen gradient). Valeric, caproic, 2-methylbutyric, isovaleric and lactic acids were also semi-quantified using the butyric acid calibration curve. Compared with BHI, Gut3Gel showed higher levels of butyric acid, lower levels of propionic acid and almost no branched-chain acids. These results show that GC-MS is the more robust choice for fatty acid analysis in Gut3Gel, and that a mucin-based matrix modifies the fatty acid profile of the microbiota. LC-MS/MS requires further optimization, including the testing of other derivatizing agents.

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Acknowledgements

The supply of samples, Gut3Gel materials and the collaboration of all of the Bac3Gel team is kindly acknowledged.

POSTER

FROM BERRY TO BOTTLE: HPLC-MS/MS MONITORING OF TFA IN NEBBIOLO GRAPES, MUST AND WINES FROM PIEDMONT

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Trifluoroacetic acid (TFA) is a highly persistent and mobile ultra-short chain PFAS that can spread through agro-food systems and be taken up by plants, but the pathways leading to its presence in grapes and wine remain poorly understood. An HPLC-MS/MS method validated for wine (Sciex QTRAP 5500, ESI−, MRM; Luna Polar Pesticides column with delay column) was applied to historical Nebbiolo wines from two Piedmont wineries (Gattinara and Asti) and to berries and must from the winery producing the Gattinara wine, obtained from the same grapes used for vinification [1]. Berries were sampled every ~20 days (20 June–18 September 2026) and stored at −80 °C; 1 g of crushed berries was extracted with water/methanol (50:50, v/v) and centrifuged, and three aliquots were analysed. Must was produced from the same sampled grapes, harvested mostly around 30 September. In Asti, TFA showed marked interannual variability and an increasing trend, with a maximum of 407.1 µg/L in 2022. In Gattinara, the 2002–2012 vintages were close to background (mean 14.6 µg/L), whereas from 2014 onwards concentrations were markedly higher (mean 120.1 µg/L; range 77.1–168.7 µg/L), consistent with increases reported in other European countries [2]. Berries contained 14.3–59.4 ng/g with no consistent seasonal pattern; replicates were repeatable (RSD 1.0–19.9%), whereas independent samples from the same bunch differed by 1.3- to 2.8-fold. A must sample contained 151.9 ± 16.3 ng/g, 4.5- to 10.7-fold higher than berries analysed in the same session. Low TFA levels were also found in surface soil and cork extracts. These data reveal distinct, site-specific temporal patterns in wine; the differences between berries, must and wine warrant further investigation, and the sources of TFA cannot be identified from these data.

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