METABOLIC FOOTPRINTING OF RESPONSE TO TREATMENT IN EX VIVO OVARIAN CANCER MODELS

R Mendes[1,2,*], G Graça[3], F Silva[4], ACL Guerreiro[1,2], P Gomes-Alves[1,2], A Félix[4,5], C Brito[1,2], IA Isidro[1,2]

[1]iBET, Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal; [2]Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal; [3]Division of Integrative Systems Medicine and Digestive Disease, Faculty of Medicine, Imperial College London, UK; [4]CEDOC-FCM-NOVA, Portugal; [5]IPOLFG, Portugal

*presenting author: rita.mendes@ibet.pt

BACKGROUND

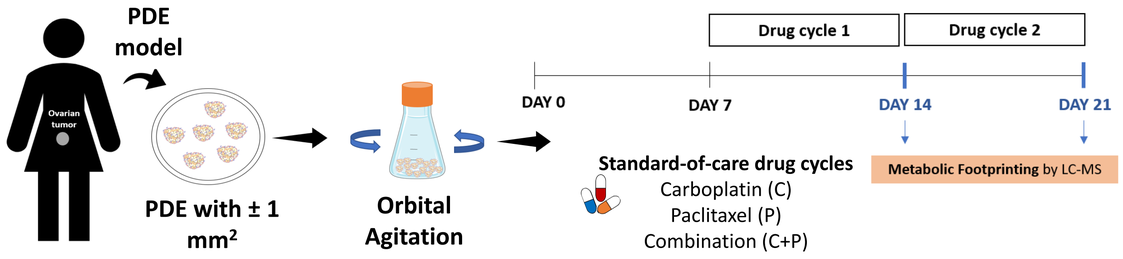
Diagnosing chemoresistance and predicting patient outcome is still a major challenge in oncology, particularly in ovarian carcinoma (OvC)[1]. Tumor progression and response to treatment is deeply influenced by the complex cell-cell, cell-extracellular matrix (ECM) and cell-soluble factor interactions, including metabolites, that compose the tumor microenvironment (TME)[2,3,4]. Alterations in cellular metabolism are a hallmark of cancer cells and sensitivity to treatment has been correlated with these alterations[5]. Importantly, the role of tumor cell-extrinsic microenvironmental factors is increasingly recognized as a modulator of the metabolic phenotype of cancer cells. Thus, untargeted metabolomics can uncover complex metabolic signatures of drug treatment responses, which could be correlated with treatment efficacy and extremely valuable to investigate [6]. We have recently developed an OvC patient-derived explant (PDE) model, which retains features of the original TME[7] and allow us to perform ex vivo drug assays simulating the clinical treatment cycles.

MAIN GOAL

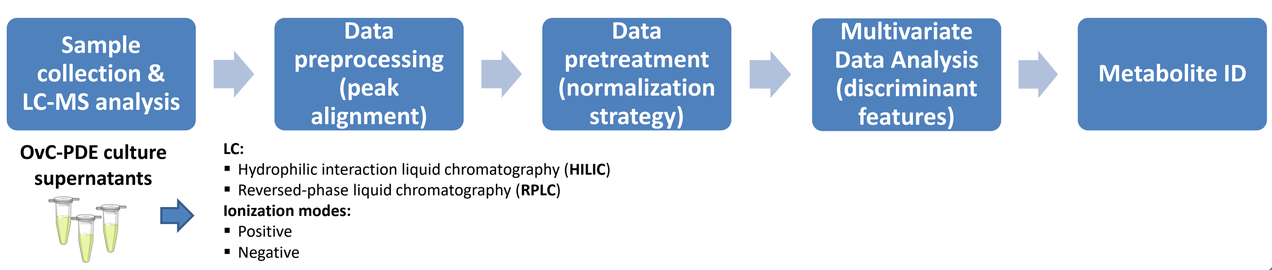
Development of a **framework for metabolomics** using **OvC-PDE models** to uncover **potential biomarkers** to facilitate **therapy assignment** in a clinical setting.

STRATEGY WORKFLOW

OvC-PDE culture establishment



Untargeted metabolomic analysis

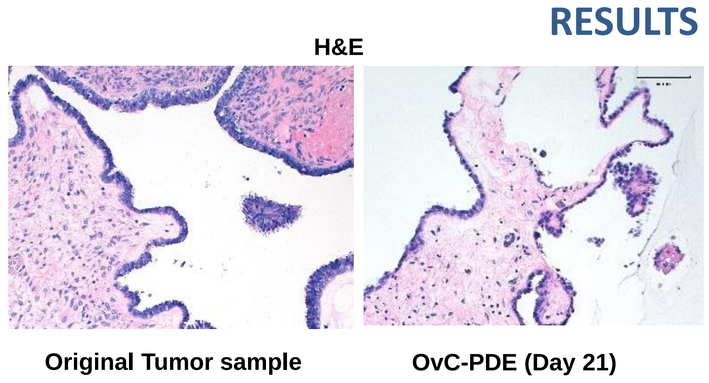


RESULTS

1

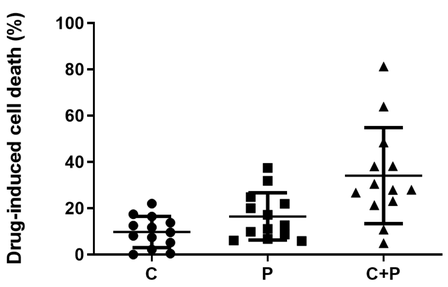
Age †	Cases (N = 13)	%
<60 years	5	38
≥60 years	8	62
Histopathology diagnosis	**Cases (N = 13)**	**%**
High-Grade Serous Carcinoma*	8	61.5
Mucinous Borderline Tumor	1	7.7
Low-Grade Serous Carcinoma**	2	15.4
Borderline Low-Grade Serous Carcinoma	1	7.7
Adult Granulosa Cell Tumor	1	7.7

† age range 25-85 years, Median age 62 years
*2 and **1 case with chemotherapy treatment before surgery.



✓ **OvC-PDE** long-term dynamic cultures **retained features of the original tumor**: preserve cell viability, tumor architecture and cell type heterogeneity for different OvC types[7].

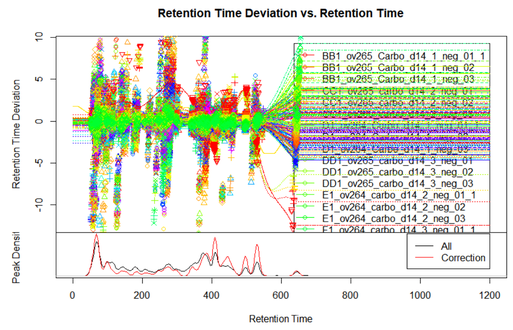
2



✓ **OvC-PDE response** to cyclic drug exposure was **sample-specific**: in general, carboplatin induced the lowest cytotoxic response, followed by paclitaxel and the combination. The latter presents the highest variability among cases.

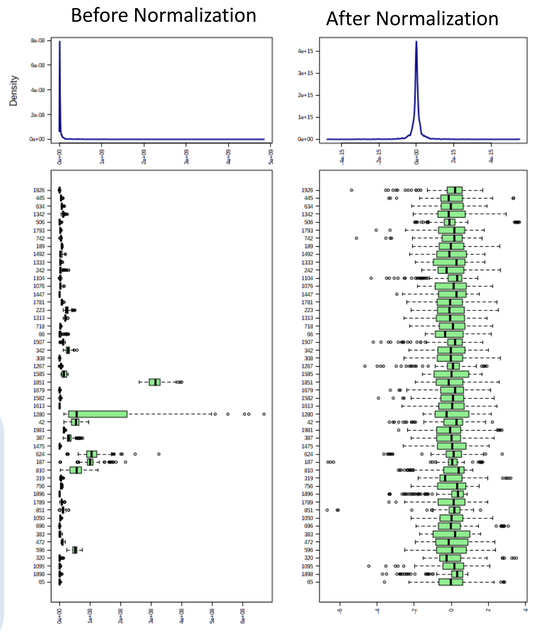
3

Data preprocessing



Example shown for HILIC column in the negative ionization mode.

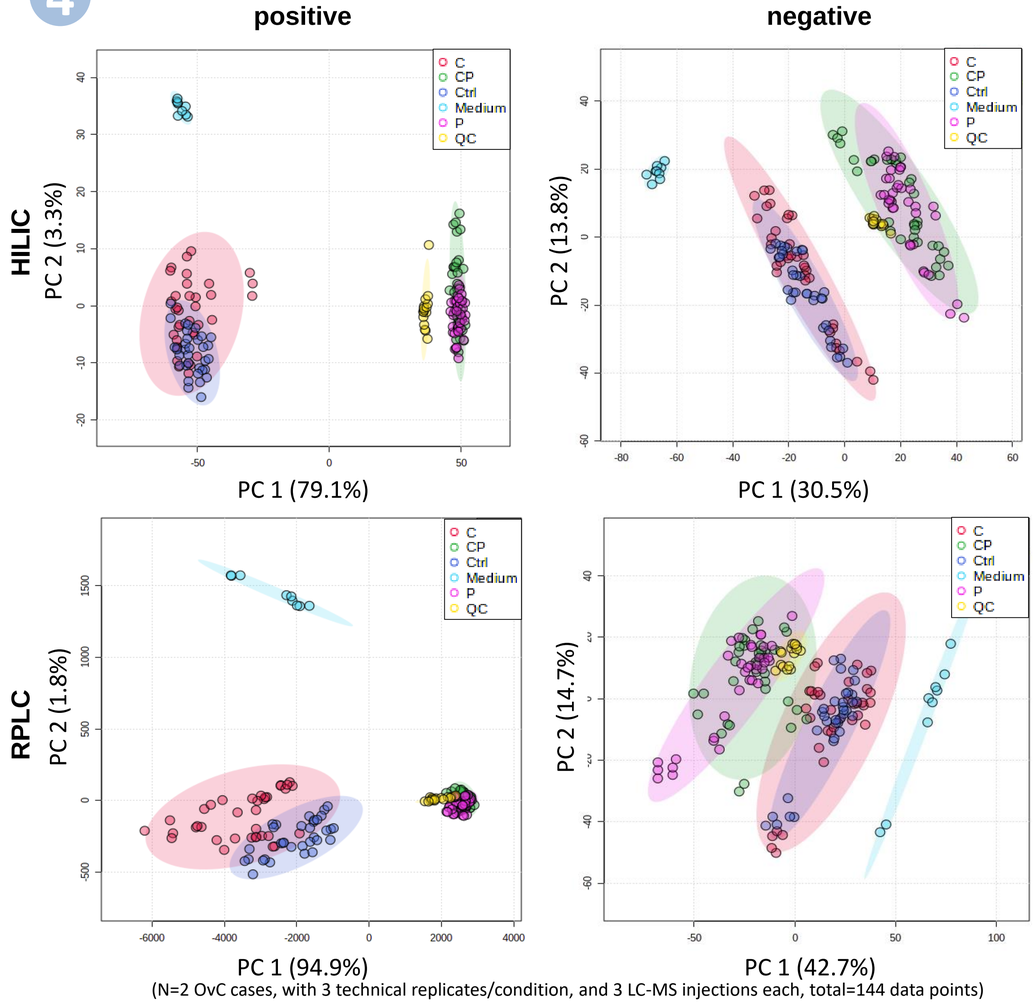
Data pretreatment



Example shown for HILIC column in the negative ionization mode.

✓ The **correction of retention time drifts** (left) and **normalization** of peak intensities across samples and features (right) was performed. After this **features become comparable**, with boxplots symmetric and of similar range (right).

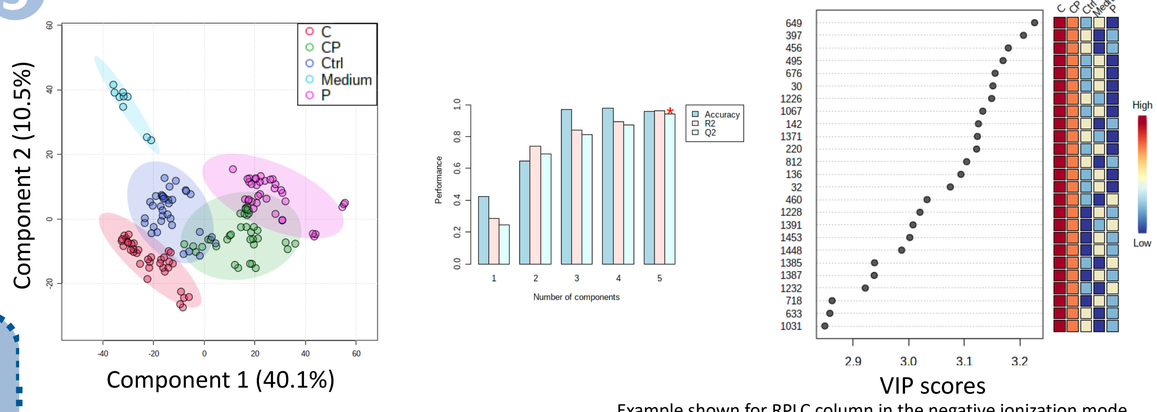
4



(N=2 OvC cases, with 3 technical replicates/condition, and 3 LC-MS injections each, total=144 data points)

✓ PCA of **metabolic footprints** revealed **different drug response groups** in both columns and ionization modes tested, supporting the existence of characteristic metabolic signatures. The cluster of quality control (QC) data points indicates adequate performance of the analytical and data processing platform.

5



Example shown for RPLC column in the negative ionization mode.

✓ PLS-DA of **metabolic footprints** found **discriminant features between drug treatment groups** for subsequent identification of corresponding metabolites: a panel of 25 metabolic features was selected by VIP score

CONCLUSION AND FUTURE WORK

Overall, this study provides a **proof-of-concept** for **drug response evaluation** by **metabolic footprinting**, paving the way to uncover potential biomarkers of drug response and resistance. Currently, we are focused on the identification of the discriminant features among treatment groups.

References: 1. Matulonis et al, Nat Rev Dis Primers, 2016, doi: 10.1038/nrdp.2016.61; 2. Santo et al, Biotechnol J, 2016, doi: 10.1002/biot.201600505; 3. Pogge von Strandmann et al, Trends in Cancer, 2017, doi: 10.1016/j.trecan.2016.12.005; 4. Luo et al, Cancer Lett, 2016, doi: 10.1016/j.canlet.2016.04.038; 5. Zaal & Berkers, Front Oncol, 2018, doi: 10.3389/fonc.2018.00500; 6. Lau & Vander Heiden, Annu Rev of Cancer Biology, 2020, doi: 10.1146/annurev-cancerbio-030419-033333; 7. Abreu et al, Sci Rep, 2020, doi: 10.1038/s41598-020-76291-z

Cofinanciado por:

Acknowledgements:
iNOVA4Health – UID/Multi/04462/2020, funded by FCT/MEC; Abbvie; FCT - SFRH/BD/132163/2017, PD/BD/128377/2017.
ER Boghaert is acknowledged for insightful discussion and for sharing his expert opinion