Fast-Tracking Cancer Metabolism Research: Integrating Discovery and Targeted Metabolomics for Comprehensive Biological Insights

Crystal L. Pace[1, 2]; Wen-Hsuan Chang[3]; Clint A. Stalnecker[2, 3]; Channing J. Der[2, 3]; Erin Redman[4]; J. Will Thompson[5]; Laura E. Herring[1, 2]; Whitney L. Stutts[1, 2]

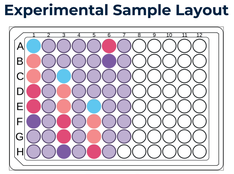
[1]UNC Metabolomics & Proteomics Core, University of North Carolina at Chapel Hill, Chapel Hill, NC; [2]Department of Pharmacology, University of North Carolina at Chapel Hill, Chapel Hill, NC; [3]Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC; [4]Repligen Corporation, Morrisville, NC; [5]Move Analytical LLC, Carrboro, NC

INTRODUCTION

Metabolomics offers exciting opportunities to uncover important biological mechanisms through advanced analytical methodologies and instrumentation. Here, we highlight a hybrid metabolomics workflow that integrates targeted analysis and discovery metabolomics. Using capillary-electrophoresis coupled to high-resolution mass spectrometry, we achieved high sensitivity and robust quantitation of over 100 analytes, while also collecting untargeted data. This dual capability allows for quantitating known metabolites and identifications of new molecular features from discovery-based analyses. We applied this methodology to pancreatic tumor xenografts and plasma from mice treated with a novel combination therapy approach targeting the KRAS oncogene and the metabolic enzyme glutaminase. Additionally, we introduce a refined targeted method using the Stellar mass spectrometer, reducing method development time from weeks to days through ultra-fast acquisition and diverse scan modes.

EXPERIMENTAL DETAILS

Experimental Sample Layout

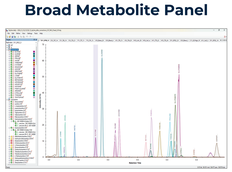


Process Blanks, Calibration Standards, Quality Control (QC) Samples, Study Pooled QCs, Experimental Samples

ZipChip HR – Fusion Lumos

Data-Dependent Acquisition

Broad Metabolite Panel



Data Processing & Visualization in Skyline

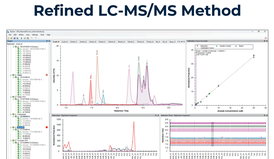
Discovery Pipeline

Compound Discoverer Integration and Annotation

Targeted PRM Development

HILIC Chromatography on Stellar Mass Spectrometer

Refined LC-MS/MS Method



Absolute Quant of TCA Cycle, Glycolysis, and Amino Acids

Figure 1. Metabolites were extracted from mouse plasma (20 µL; n=3/group) and tumor xenografts (20 mg; n=3/group) by methanolic protein precipitation with stable isotope-labeled internal standards. Tumor xenografts were weighed, homogenized, and further diluted in deep-well plate to achieve 0.5 mg tissue per sample. Quality Control, Calibration, and Blank Samples were prepared alongside experimental samples. After filtered centrifugation into deep-well plates, samples were analyzed using microchip capillary-electrophoresis coupled to an Orbitrap Fusion Lumos Tribrid MS with data-dependent acquisition. Raw files were processed using a Skyline v24.1 template containing target molecules with an in-house built algorithm for peak assignment correction. Single-point quantitation was performed using a validated matrix-based calibration material. Further data processing in Compound Discoverer allowed for the putative identification of additional molecules significantly altered between treatment groups. The combined discovery and quantitative data from the Broad Metabolite Panel highlighted additional metabolites of interest for further investigation. Utilizing the Stellar mass spectrometer, a targeted LC-MS/MS method was developed and optimized to provide absolute quantitation of these analytes.

BROAD METABOLITE PANEL

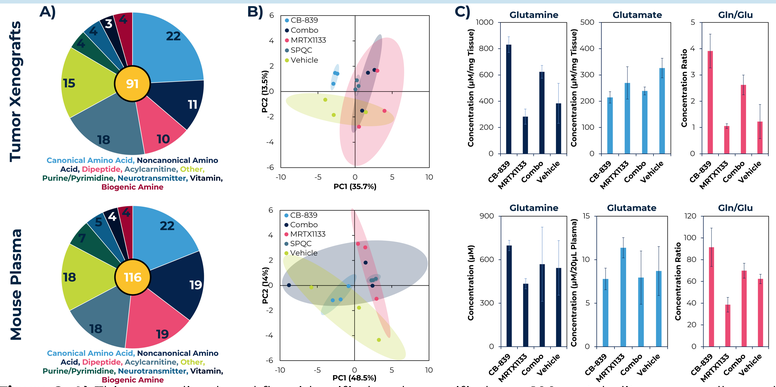


Figure 2. A) This streamlined workflow identified and quantified over 100 metabolites across all sample matrices and experimental conditions. **B)** PCA showed distinct separation of CB-839 from other treatment groups in tumor xenografts with less separation observed in mouse plasma. **C)** Concentrations of glutamine increased with CB-839 treatment, while glutamate decreased with CB-839 treatment. Observed differences were statistically significant in tumor xenografts and insignificant in mouse plasma. Relative to glutamine, glutamate showed statistically significant differences between treatment groups in both tumor xenografts and mouse plasma samples.

DISCOVERY METABOLOMICS

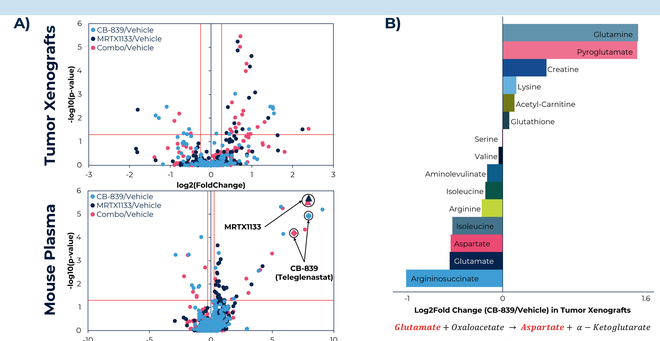


Figure 3. A) Global view of dysregulated features between treatment groups. Notably, the drug treatments were identified in their respective treated samples which was crucial to verify successful drug delivery. This approach enabled the identification of other key metabolites, providing insights into biological variations between treatment groups. **B)** Many dysregulated analytes were metabolites that are involved in the metabolism of glutamine, some of which have an effect on the TCA cycle.

TARGETED ANALYSIS ON STELLAR MS

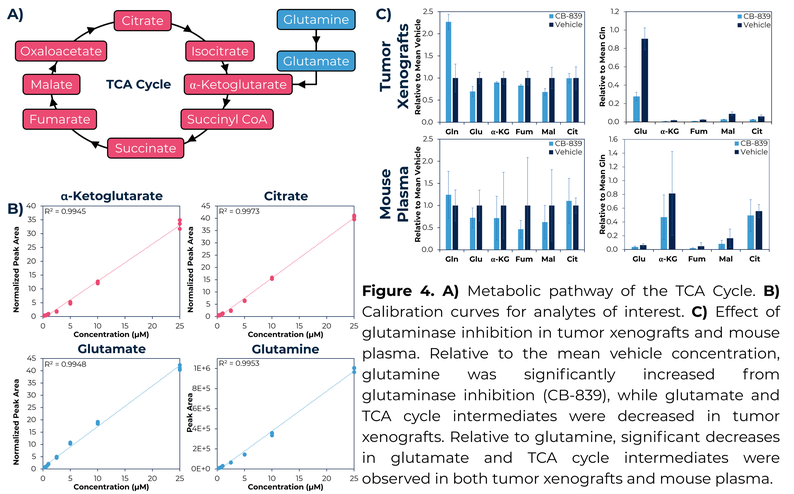


Figure 4. A) Metabolic pathway of the TCA Cycle. **B)** Calibration curves for analytes of interest. **C)** Effect of glutaminase inhibition in tumor xenografts and mouse plasma. Relative to the mean vehicle concentration, glutamine was significantly increased from glutaminase inhibition (CB-839), while glutamate and TCA cycle intermediates were decreased in tumor xenografts. Relative to glutamine, significant decreases in glutamate and TCA cycle intermediates were observed in both tumor xenografts and mouse plasma.

CONCLUSIONS

- The Broad Metabolite Panel allowed for quick and efficient extraction and quantitative analysis of metabolites (**Figure 1**).
- More than 100 metabolites were identified across mouse plasma and tumor xenografts (**Figure 2A**).
- Effects of glutaminase inhibition (CB-839) was observed by increased glutamine and decreased glutamate in tumor xenografts (**Figure 2C**).
- Data dependent acquisition of the Broad Metabolite Panel allowed for the additional discovery workflow in Compound Discoverer, enabling the identification of additional features of interest to target in future studies (**Figure 3**).
- TCA cycle intermediates and amino acids were targeted by an LC-MS/MS method developed on the Stellar MS which demonstrated the downstream effects from glutaminase inhibition (**Figure 4**).

ACKNOWLEDGEMENTS & CONFLICT OF INTEREST

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