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TITLE
Global and Targeted Metabolic Profiling Identifies Metabolic Markers for FLT3-ITD in Pediatric AML Patients

HYPOTHESIS:
Plasma samples from FLT3/ITD pediatric AML patients will show a significant difference in metabolite abundance, overall metabolic profile, and metabolic pathway activity when compared to plasma samples from FLT3 wild type pediatric AML patients.

BACKGROUND/AIMS:
Acute myeloid leukemia (AML) is a clinically challenging disease with high interpatient variability in response to chemotherapy. Despite continuing advances in treatment options, current 5-year survival rates for pediatric AML are suboptimal at ~60%. Variability in treatment response and survival outcomes are due in part to the heterogeneous nature of AML, with many genetic lesions and cytogenetic features contributing to disease progression. One of the most well known genetic lesions associated with AML involves Fms-Like Tyrosine Kinase-3 (FLT3), a receptor tyrosine kinase expressed in hematopoietic stem cells. Internal tandem duplication of the juxtamembrane domain coding sequence of FLT3 (FLT3/ITD) causes autonomous cellular proliferations leading to disease progression. Previous metabolomics studies have successfully identified significant metabolic alterations in hematological malignancies. However, no metabolomics studies on pediatric AML have been reported at this time. In this study, we propose to use global and targeted metabolomics to identify differential metabolite abundance, metabolic profile, and pathway activity associated with FLT3-ITD status in pediatric AML patients treated in the St Jude AML02 clinical trial.

METHODS:
Serum metabolomics profiles were generated with serum samples obtained at diagnosis from patients treated in the St. Jude AML02 study. Patients were assigned to FLT3 Wild Type (n=13) and FLT3-ITD (n=59) groups. Global metabolomics profiling was performed on a Thermo Q-Exactive Orbitrap mass spectrometer with Dionex UHPLC and autosampler. Targeted metabolomics profiling was generated for a select group of organic acids and acylcarnitines. The organic acid panel included eight metabolites related to the tricarboxylic acid cycle and glycolysis. The acylcarnitine panel will feature 57 varieties of acylcarnitine. Metabolomics profiling was performed on an Agilent 6490 triple quadrupole with an Agilent 1290 HPLC. Absolute quantification was achieved through external comparison on standard curves generated for each targeted metabolite on the organic acid and acylcarnitine panels. Univariate and multivariate analyses were performed using MetaboAnalyst web based software.

RESULTS & CONCLUSIONS
Statistical analysis on metabolomics data identified 17 known metabolites significantly associated with FLT3 status (p<0.05). Pathway enrichment analysis identified 22 metabolic pathways significantly impacted by difference in FLT3 status. Organic acid targeted analysis identified four organic acids with significantly different abundance associated with FLT3 status (p<0.05) and 24 metabolic pathways significantly impacted by difference in FLT3 status (p<0.05). Acylcarnitine targeted analysis identified two acylcarnitines (octadecanoylcarnitine and hexanoylcarnitine) significantly associated with difference in FLT3 status. Overall, this study identifies several metabolites and metabolic pathways significantly associated with the FLT3-ITD status in pediatric AML patients. These results help expand on previously conducted pilot studies and further clarify the metabolic differences associated with the FLT3-ITD form of AML. Ideally, continued metabolic profiling of additional AML subtypes can reveal pathways and networks that can be used to improve the efficiency of AML diagnosis and risk evaluation.