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Early Phospho-Signaling Shifts in Smart Buffer™-Preserved Peripheral Blood Specimens in Response to FLT3 Inhibitor Therapy

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Abstract

Introduction

Identification of druggable mutations in Acute Myeloid Leukemia (AML), such as FLT3-ITD, has led to the development of targeted therapies capable of out-performing cytotoxic chemotherapy. Despite early survival benefits, most patients with FLT3-ITD AML on trial with the FLT3 inhibitor (FLT3i) gilteritinib still develop treatment resistance. Critical readout of response to FLT3i as measured by phospho-signaling is missing for the majority of these patients. Here, we validated an innovative approach to obtaining these signatures by leveraging the Smart Buffer™ preservation system for peripheral blood (PB) specimens integrated with phospho-mass cytometry as part of the ongoing BeatAML Trial.

Methods

PB isolates were collected at five sites and preserved in Smart Buffer™, an intracellular signaling stabilizer, prior to freezing and shipping to OHSU. Matched Day 1 and Day 4 samples (N=14) from seven patients recently initiated on gilteritinib were thawed and barcoded with palladium isotopes prior to staining with the Maxpar Human AML Phenotyping Panel (Fluidigm) and phospho-specific antibodies to targets in the FLT3 signaling cascade (pERK, pSTAT5, pAKT, pS6, and pFLT3). Samples were run on a Helios mass cytometer with downstream debarcoding, compensation, and data visualization achieved with the CATALYST R package.

Results

Evolution in aggregated phospho-signaling profiles occurred in all paired isolates. FlowSOM clustering and tSNE analysis showed that the highest levels of phospho-signaling were captured within $CD45^{low}CD34^{high}$ blast populations. Median expression of each phospho-protein was heterogeneous at a baseline in patients, but largely reflected

decreases in Day 4 profiles relative to Day 1. Statistically significant decreases in blast signaling were found in pSTAT5, pFLT3, and pERK.

Conclusions

In paired unstimulated PB samples stabilized in Smart Buffer™, detectable changes in phospho-signaling can be obtained through mass cytometry. These metrics may offer an easily obtainable orthogonal readout of treatment responses to targeted therapies such as FLT3i in patients with AML.