SOHC
SWISS ONCOLOGY & HEMATOLOGY CONGRESS

Inflammation-mediated resistance to FLT3 inhibition in acute myeloid leukemia

Peppi Suominen[1], Krithika Rajeeth[1], Paul Gueguen[2], Monika Burocziova[1], Jeremy Deuel[1], Stefan Balabanov[1], Markus G. Manz[1], Xufeng Chen[3], César Nombela-Arrieta[1], Jana M. Ellegast[1]

[1] University and University Hospital Zurich, Faculty of Medicine, Department of Medical Oncology and Hematology, Zurich. [2] Functional Genomics Core Zurich, ETH Zurich, Zurich.
[3] Department of Hematopoietic Biology and Malignancy, Division of Cancer Medicine, The University of Texas MD Anderson Cancer Center, Houston, Texas.

Introduction

FMS-like receptor tyrosine kinase 3 (FLT3) is the most frequently mutated gene in acute myeloid leukemia (AML). FLT3 inhibitors have improved patient outcomes but resistance limits durable disease control. Chronic inflammation is linked to cancer drug resistance, but longitudinal studies at single-cell resolution tracking the rewiring of inflammatory pathways in matched diagnostic and relapsed samples from AML patients are limited. Here, we used orthogonal models (**Figure 1**) to map the dynamics of inflammatory signaling under the selective pressure from FLT3 inhibition.

Methods/Results

We established gilteritinib-resistant MV4-11 AML cell lines by continuous exposure of increasing doses of gilteritinib (gil), a second-generation FLT3 inhibitor (**Figure 2A**). The cells were cross-resistant to midostaurin, a first-generation FLT3 inhibitor, suggesting a common resistance mechanism among FLT3 inhibitors (**Figure 2B**). Gene set enrichment analysis revealed altered inflammatory signaling in the resistant cell line models, with TNFα-NFκB signaling as the most prominently altered pathway (**Figure 3**).

Consistently, in a matched diagnostic-relapse pair from a FLT3 mutated patient treated with a FLT3 inhibitor, the relapse sample showed enrichment of TNFα-NFκB signaling (**Figure 4 and Figure 5**). Notably, altering the timing or nature of the treatment affected resistance dynamics and led to distinct changes in inflammatory signaling, underscoring heterogeneous resistance trajectories.

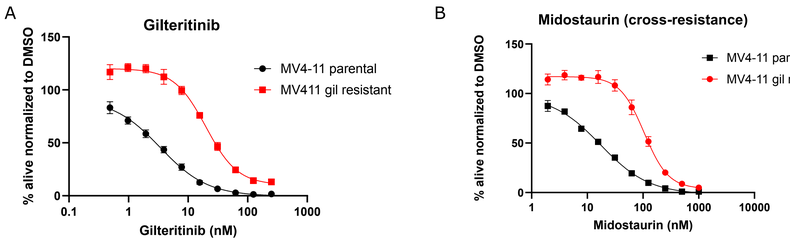

Figure 2. Dose-response assays of drug resistant cell lines. To determine IC50 values of parental and resistant MV4-11 cell lines, the cells were treated with increasing doses of FLT3 inhibitors, incubated for 72 hours and viability was measured using CellTiter-Glo One and a plate reader. **A)** Response to gilteritinib. **B)** Response to midostaurin.

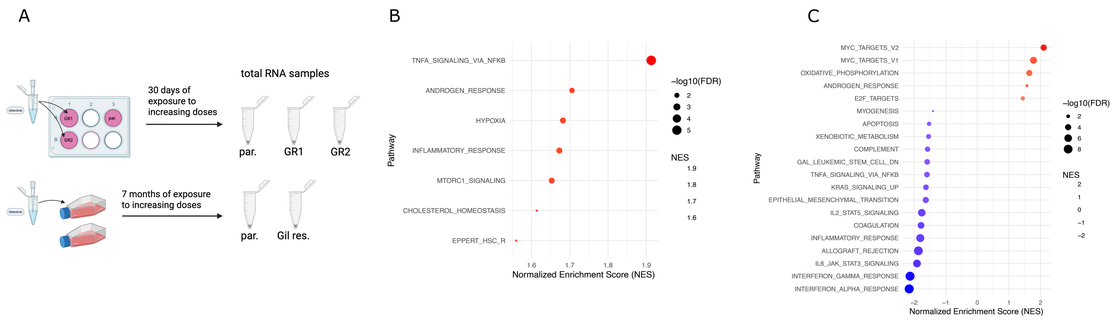

Figure 3. Pathway enrichment analysis of gilteritinib-resistant MV4-11 cell lines. A) Illustration of two independent gilteritininb resistant MV411 cell line models. First model was exposed to increasing doses of gilteritinib for 30 days and second model for 7 months. **B)** Enriched pathways in gil resistant cells exposed to gil for 30 days. Gene set enrichment analysis (GSEA) was performed using MSigDB Hallmark and leukemic stem cell gene sets. **C)** Enriched pathways in gil resistant cells that were exposed to gilteritinib for 7 months.

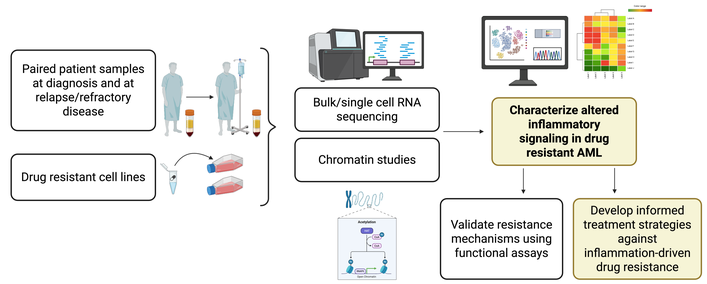

Figure 1. Research strategy. Two complementary models—paired patient samples from diagnosis and refractory or relapsed AML, and drug-resistant cell lines were to characterize inflammatory signaling in drug-resistant AML through gene expression and chromatin studies. These data will be used to generate hypotheses on resistance mechanisms and guide the development of treatment strategies against inflammation-driven drug resistance. The hypotheses will be functionally tested *in vitro*.

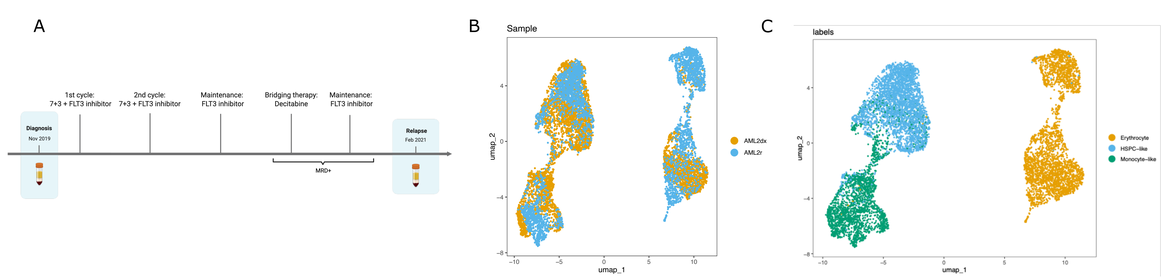

Figure 4. Case study of a patient who relapsed on FLT3 inhibitor–based therapy. A) Clinical timeline of a relapsed AML patient, including diagnosis, treatment, and relapse. Bone marrow aspirates were collected at diagnosis and relapse and stored at the USZ biobank. **B)** UMAP showing single-cell transcriptomic profiles of diagnostic and relapsed samples. **C)** UMAP annotated with cell types in both samples.

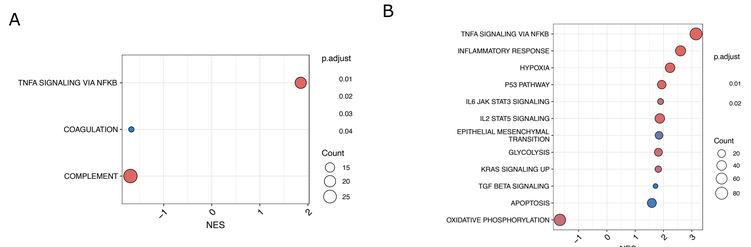

Figure 5. Pathway enrichment in AML relapse. A) Gene set enrichment analysis in hematopoietic stem and progenitor (HSPC) -like cells. **B)** Gene set enrichment analysis in monocyte-like cells. Positive normalized enrichment scores (NES) indicate upregulation in the cells from the sample collected at disease relapse.

Conclusions

- Resistance to FLT3 inhibition is associated with transcriptional rewiring of inflammatory signaling pathways.
- Drug-resistant AML cell line models reflect gene expression changes observed in sequential patient samples and are thus valuable models to study resistant disease states.
- Altering duration and intensity of a defined selective pressure might distinctly influence particular inflammatory signals.

Presented at SOHC 2025 from 19 – 21 November 2025