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Targeting autophagy enhances Midostaurin's impact on FLT3-mutated leukemia stem cells under conditions mimicking hypoxic niche

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INTRODUCTION

The *FLT3-ITD* mutation is among the most recurrent alterations and predicts for a dismal prognosis in acute myeloid leukemia (AML). [1] The tyrosine kinase inhibitor Midostaurin (Mido) has been approved in association with chemotherapy for newly-diagnosed *FLT3*-mutated AML patients. While Mido demonstrates clinical effectiveness, it falls short in eliminating LSCs, leading to limited responses and eventual resistance development. [2] The hypoxic niche, where LSCs physiologically reside, acts as a protective environment, sustaining various pro-survival mechanisms crucial for AML development and treatment response.[3]

AIM

The study aimed to investigate the metabolic and transcriptomic changes in *FLT3*-mutated AML cells treated with midostaurin under conditions mimicking the hypoxic niche. The final goal has been the identification of novel combination strategies to eradicate LSCs which reside in hypoxic niches.

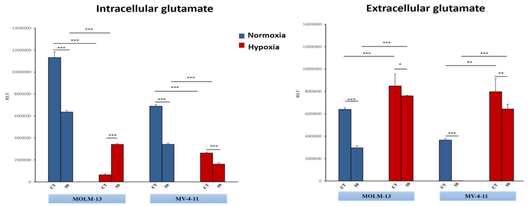
METHOD

MOLM-13 and MV-4-11 *FLT3*-mutated (*FLT3*-mut) AML cell lines were incubated for 16h in normoxia and hypoxia (1% O_2) , then treated with Mido (100nM) and the autophagy-inhibitor chloroquine (Cq; 10 uM), as single agent or in combination for up to 24h. Metabolic perturbations were explored by glutamate and ROS-glo reagents (Promega). Transcriptomic alterations were analyzed by gene expression profiling (GEP, Affymetrix), while gene set enrichment analysis (GSEA) was used to identify deregulated pathways. Percentage of apoptotic cells was detected by AnnexinV/PI staining and analyzed by CytoFlex flow cytometer (Beckman).

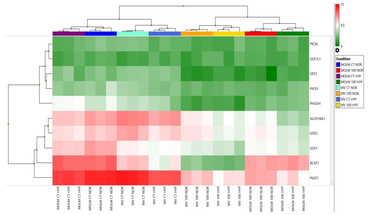
RESULTS

1- Glutamate levels are affected by Mido treatment

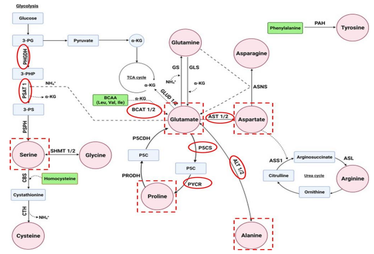
Mido triggers a pronounced decline in both extracellular and intracellular glutamate levels both in MOLM-13 and in MV-4-11 cell lines.



2- Mido suppresses the expression of genes crucial for non-essential amino acid (NEAA) synthesis

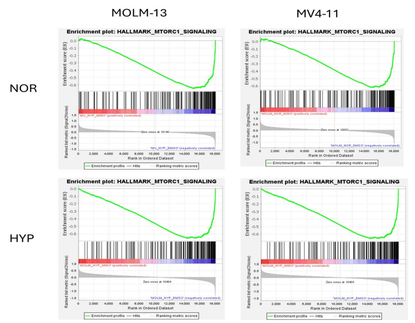


Mido down-regulates the expression of genes that code for biosynthetic enzymes crucial for glutamate conversion into diverse NEAA.



Graphic representation of metabolic alterations induced by Mido treatment. Enzymes encoded by down-regulated genes (circled in red) are involved in the synthesis of alanine, proline, serine and aspartate.

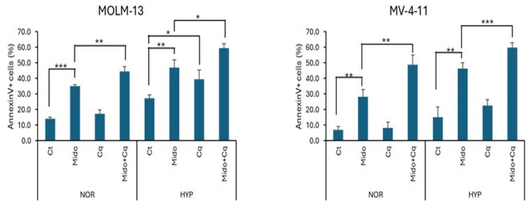
3- Mido down-regulates mTORC1 pathway



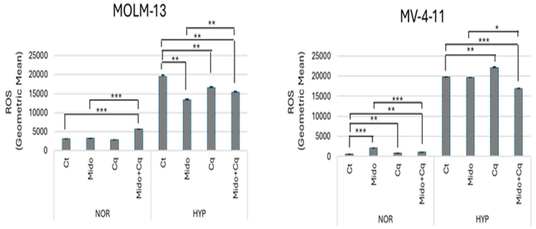
GSEA of down-regulation of a mTORC1 signature obtained in MOLM-13 and MV4-11 cell lines after Mido exposure under normoxic and hypoxic conditions.

4- Concurrent treatment with the autophagy inhibitor chloroquine (Cq) synergistically enhances the antileukemic effects of Mido and influeces ROS production

Co-treatment with Mido and Cq significantly increased the percentage of AnnexinV+ apoptotic cells in both *FLT3*-mut cell lines under normoxia and hypoxia, compared with single agent effects.



The combination of Mido and Cq significantly reduced reactive oxygen (ROS) levels under hypoxic culture conditions, compared with control and single agent treatment.



CONCLUSIONS

Integration of metabolic and transcriptomic data highlights the activation of the autophagy network as a potential pro-survival mechanism adopted by *FLT3*-ITD mutated cells after Mido exposure. Our results showed that treatment induced mTORC1 pathway inhibition and non essential amino acid (NEAA) deprivation, both associated with autophagy induction. Consistent with this finding, concurrent treatment with the autophagy inhibitor chloroquine synergistically enhanced the antileukemic effects of Mido, leading to decreased viability.

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