Dual CXCR4 and E-Selectin Inhibition (GMI-1359) Rapidly Increases AML Cellular Motility Prior to Intravasation and Vascular Niche Depletion Observed by Intravital Bone Marrow 2-Photon Microscopy

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The bone marrow (BM) microenvironment contributes to leukemia therapy resistance.

Acute myeloid leukemia (AML) cells home to BM through adhesive and chemokinetic interactions, especially by sialylated glycoproteins on AML cells binding to E-selectin on endothelial cells (EC) and by CXCR4 sensing of the SDF-1/CXCL12 chemokine (Chien 2013; Peled and Tavor 2013).

A next generation “mobilizing” agent GMI-1359 combines in one molecular structure two antagonist moieties for E-selectin and CXCR4.

In a previous study, GMI-1359 markedly reduced leukemia cell adhesion to ECs and cellularity in BM (Zhang 2016) and it enhanced survival in cytarabine/daunorubicin therapy model (Zhang 2015).

Exactly how AML cells behave in response to dual E-selectin and CXCR4 inhibition in living BM remains unclear.
GMI-1359 Mobilizes Leukemia Cells into Peripheral Blood Faster than a Single CXCR4 Inhibitor Plerixafor

Weiguo Zhang et al.  
ASH2020 Abstract 2865
Imageable Syngeneic p53-null AML Model

2-photon microscope

AML-mTurq2 cells I.V.

C57BL6 fluorescence reporter mice

Surgical preparation

Genetics:
• p53-/-
• MLL
• ENL-FLT3 ITD
• (mTurquoise2)

Peripheral Blood, Day 14

Calvarial BM filled with AML cells (blue)

In vivo mouse skull 2-photon fluorescence, 4x
AML cells are migratory in BM

STEADY STATE

Time lapse: 20 min

AML Blood Host CD11c cells

BM AML Cell Mean Speed

p<0.001 (One-way ANOVA)

longitudinal time from GMI1359 IV
(Whiskers: 95% CI)
GMI-1359 increases AML cell motility in BM

20min AFTER GMI-1359 I.V. (same field of view)

Time lapse: 1 h

AML Blood Host CD11c cells

BM AML Cell Mean Speed

p<0.001 (One-way ANOVA)

longitudinal time from GMI1359 IV
(Whiskers: 95% CI)
Mobilization of quizartinib-induced AML minimum residual disease by GMI-1359

Quizartinib 60 mg/kg started when ~5% blasts in PB, for 10 days, resulting in >99% cytoreduction.

Calvarial bone marrow imaged in vivo by 2-photon microscopy.

GMI-1359 20 mg/kg injected IV.

Recording 0 – 6 hours after GMI-1359 injection.
GMI-1359 decreases AML cellularity predominantly in the vascular niche

**BEFORE GMI-1359**

**4h AFTER GMI-1359 (different mouse)**

AML cells are located mostly in the vascular niche: 58% of all BM AML cells

Vascular niche contains only 46% of all BM AML cells
Conclusions

• AML cells are intrinsically motile in BM stroma.

• AML cells congregate largely in the vascular niche.

• Dual E-selectin/CXCR4 inhibition greatly increases the speed of AML cell migration inside BM.

• GMI-1359 depletes AML cells in BM predominantly in the vascular niche and less so in the sites proximal to the bone.

• Further investigation should focus on the molecular mediators of AML persistence in the bone niche and on ways to target leukemia cells in this niche.