Co-targeting E-selectin/CXCR4 with GMI-1359 Facilitates AML Stem Cell Mobilization and Protects BM Niches from Anti-leukemia Therapy

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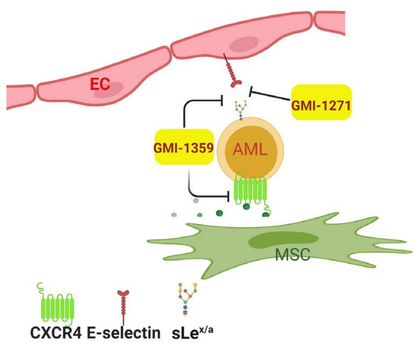
Introduction

Acute myeloid leukemia (AML) cells compete with normal hematopoietic cells and rewire the bone marrow (BM) microenvironment into niches that selectively support leukemia stem cells (LSC). The vascular adhesion molecule, E-selectin is expressed on endothelial cells (EC) in the perivascular niche where therapy-resistant AML cells have an increased affinity to E-selectin compared to normal hematopoietic stem cells (HSC) (Winkler et al., 2020). We previously demonstrated (Chang et al., ASH 2020) that E-selectin blockade by the pharmacological antagonist, GMI-1271 (GlycoMimetics, Inc) sensitized therapy-resistant LSC to Bcl-2 targeted therapy. Efficacious eradication of LSC in the BM however requires blocking multiple receptors and/or associated signaling pathways. A more optimal dislodgement of LSC from the BM could be attained by combining an E-selectin antagonism with blockade of the CXCR4/SDF-1α axis. The dual antagonist of E-selectin and CXCR4, GMI-1359 (GlycoMimetics, Inc.), has been tested in a phase 1 clinical trial (NCT02931214). Our study aims to examine a hypothesis-driven combinatorial therapy using GMI-1359 with venetoclax/hypomethylating agents (HMA) to achieve improved remission durations and reduced relapse rates for therapy-resistant patients with AML.



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Hypothesis

Co-targeting E-selectin/CXCR4 more efficiently mobilizes AML cells from BM niches and synergizes with the anti-leukemia activity of venetoclax/hypomethylating agent (Ven/HMA).

Results

In Vivo 2-Photon Microscopy Demonstrates that Increased AML cell motility by Dual Inhibition of E-selectin/CXCR4

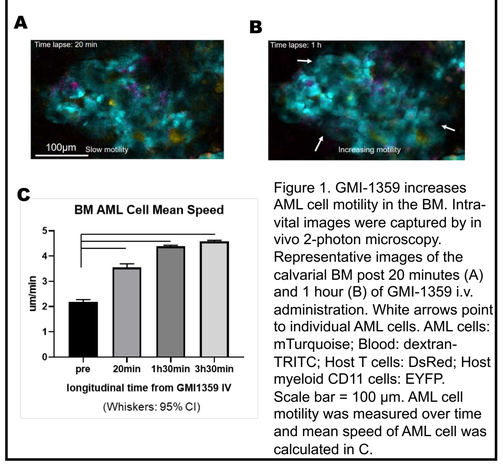

Figure 1. GMI-1359 increases AML cell motility in the BM. Intra-vital images were captured by in vivo 2-photon microscopy. Representative images of the calvarial BM post 20 minutes (A) and 1 hour (B) of GMI-1359 i.v. administration. White arrows point to individual AML cells. AML cells: mTurquoise; Blood: dextran-TRITC; Host T cells: DsRed; Host myeloid CD11 cells: EYFP. Scale bar = 100 μm. AML cell motility was measured over time and mean speed of AML cell was calculated in C.

Targeting BM-LSC Receptor/ligands in Combination with Ven/HMA Significantly Extends Survival of AML-PDX

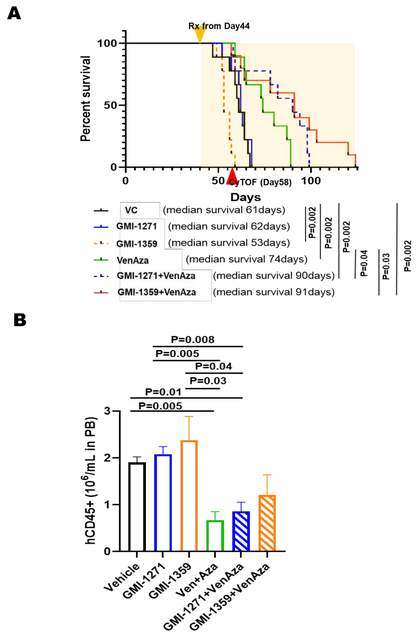

Figure 2. inhibition of E-selectin with either GMI-1271 or GMI-1359 in combination of Ven/HMA shows survival benefit. (A)Kaplan-Meier survival curves of PDX-AML (mutation: Jak2, c-Kit) mice.
(B) Absolute number of circulating AML blasts measured by flow cytometry analysis at Day 58.

Combinatorial Treatment of E-selectin Inhibitor with Ven/HMA Significantly Reduces Leukemia Burden in the BM

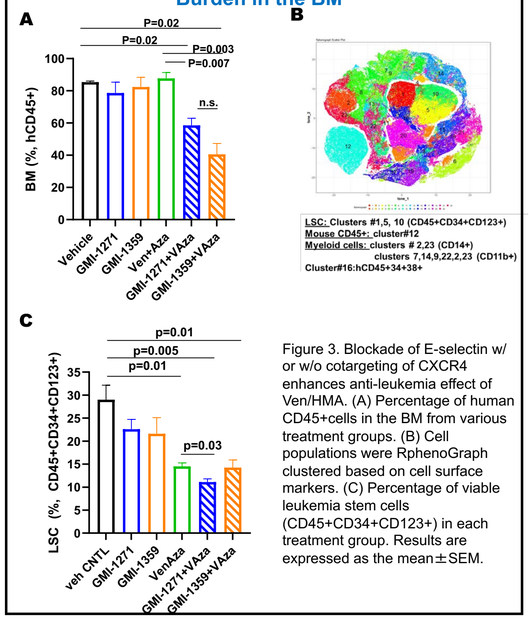

Figure 3. Blockade of E-selectin w/ or w/o cotargeting of CXCR4 enhances anti-leukemia effect of Ven/HMA. (A) Percentage of human CD45+cells in the BM from various treatment groups. (B) Cell populations were RphenoGraph clustered based on cell surface markers. (C) Percentage of viable leukemia stem cells (CD45+CD34+CD123+) in each treatment group. Results are expressed as the mean±SEM.

AML Blasts and LSC from the BM of the Combinatorial Treatment Groups Show Altered Signaling

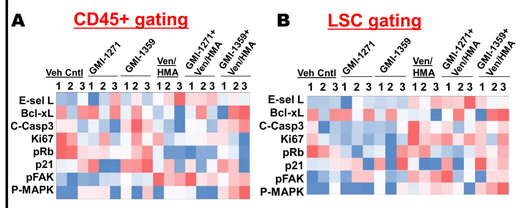

Figure 4. CyTOF analysis defines altered signaling in drug treated AML blasts and LSC in vivo. Protein levels in AML blasts (A) and LSC (B) populations in mouse BM as determined by CyTOF. The protein levels of individual samples are presented as a heatmap. BM cells were collected at D14 drug treatment (n=3 mice/group).

Targeting E-selectin/CXCR4 Protects Normal Hematopoietic Cells

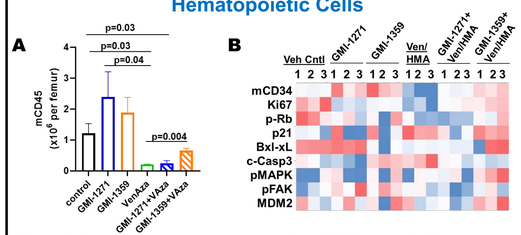

Figure 5. Normal mouse hematopoietic cells (mCD45+) in AML BM were quantified (A) and relative protein expressions were plotted in a heatmap (B)

Pharmacological Antagonist of E-selectin/CXCR4 Upregulates Survival Signaling Cascades in BM Niche Component Cells

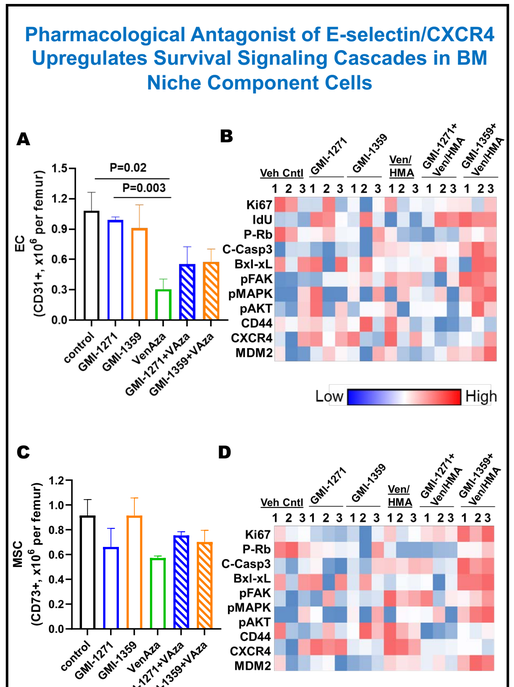

Figure 6. EC (A) and MSC (B) from the BM of PDX-AML mice (D14 drug treatment) were analyzed by CyTOF. (A) Percentage of CD31+ cells and difference in protein expressions are heatmap visualized in B. (C) Percentage of CD73+ MSC (p=n.s.) and difference in protein expressions are heatmap visualized in D. N=3 per group. Bar graphs represent mean±SEM.

Conclusions

1.Dual inhibition of E-selectin/CXCR4 increases AML cell motility in vivo.
2.Combination of E-selectin inhibition with GMI-1271 or GMI-1359 and Ven/HMA shows survival benefit in AML-PDX mice.
3.Cotargeting E-selectin (or E-selectin/CXCR4) and Bcl-2 significantly reduces BM retention of AML cells.
4.Dual antagonist of E-selectin/CXCR4, GMI-1359 protects normal hematopoietic cells in AML BM.
5.Pharmacological antagonists of E-selectin and E-selectin/CXCR4 preserve BM component cells from Ven/HMA-induced detrimental insults through upregulation of survival signaling cascades.