Combined Blockade of E-selectin and CXCR4 (GMI-1359) Enhances Anti-Leukemia Effect of FLT3 Inhibition (Sorafenib) and Protects Hematopoiesis in Pre-Clinical AML Models

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DISCLOSURE


- *Supported in part by GlycoMimetics, Inc*
• Internal tandem duplications in the fms-like tyrosine kinase 3 (FLT3-ITD) account for 24% of adult AML cases and confer poor prognosis (Thiede et al., Blood 2002; Kottaridis et al., Leukemia & lymphoma. 2003).

• FLT3 inhibitors like sorafenib only show limited efficacy in eliminating leukemia stem cells in the bone marrow (BM), which suggests a protective role for the BM niche in FLT3 mutated AML (Zhang et al., JNCI 2008; Borthakur et al.; Amer. J. Hematol. 2020).

• Homing of AML cells into BM is mediated, at least in part, by the adhesion to E-selectin on endothelial cells (ECs) and by CXCR4-directed cellular migration to stromal CXCL12 (SDF1) constituents (Chien et al., Blood 2013; Peled and Tavor, Theranostics 2013; Chen et al., J Clin Invest. 2013)

• Our previous study demonstrated that targeting E-selectin/CXCR4 with the dual E-selectin/CXCR4 antagonist GMI-1359 markedly reduced leukemia cell adhesion to ECs and mesenchymal stem cells (MSCs) in vitro, and reduced leukemia cellularity in the BM in vivo (Zhang et al., Can Res Suppl. 2016).

• GMI-1359 combined with cytarabine/daunorubicin generated a profound survival benefit in mice with FLT3-mutated leukemia (Zhang et al., Blood suppl. 2015).
HYPOTHESIS

Combinatorial targeting of CXCR4, E-selectin, and FLT3:

• Effectively mobilizes leukemia blasts and increase killing by abrogating bone marrow-mediated protection;

• Enhances survival of normal hematopoiesis.
Increased Expression of E-selectin Ligands and CXCR4 in FLT3-targeted Treatment of ITD- and/or TKD-mutated Leukemia Cells

**A**

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@ Normoxia MOLM14-96 h

**B**

![Bar chart showing increased expression of CXCR4 and CD162 under various conditions.](image)

**C**

<table>
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<th>Ba/F3-FLT3-ITD+F691L</th>
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Blockade of E-selectin/CXCR4 with GMI-1359 Reduces Leukemia Cell Adhesion, Migration, and Homing to Components of the BM Niche

**In vitro**
(MOLM14 cells)

**In vivo**
(AML mice)

Live-Cell imaging data were shown in Dr. Zal’s ASH poster #1963.
GMI-1359 Combined with Sorafenib Enhances Survival In a FLT3-ITD-mutant PDX Leukemia Model in vivo

A

Irradiation
AML cells
NSG

PDX model: I.V injection (3 x 10⁶/mouse).
AML patient sample: FLT3-ITD, WT1 mutations.

46,XY,t(15;19)(q11.2;q13.3)[7]; 46,XY[13]
Previous treatments: 3+7; HDAC(CR:4M); MEC; DAC+Soraf,E6201, CLIA+Soraf

B

Median survival was 109, 87, 126 and 138.5 days for vehicle, GMI-1359, sorafenib and combination, respectively.

* p < 0.05; ** p < 0.01.
GMI-1359 Combined with Sorafenib Reduces Leukemia Burden in a FLT3-ITD-mutated PDX AML Model *In vivo*

**A**

<table>
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</tbody>
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*anti-human CD45 antibody (green)*

**B**

![Graph showing hCD45 (%, PB) over time (Days) for different treatments.](image)

- ***p < 0.001.

![Graph showing hCD45 (%, PB) over time (Days) for different treatments.](image)
GMI-1359 Combined with Sorafenib Upregulates Normal Hematopoiesis-Associated Cytokines and Chemokines
GMI-1359 Combined with Sorafenib Increases Megakaryocytes and Myelocytes in mice BM

Hematogenesis in mouse BM

** $p < 0.01$; *** $p < 0.001$. 
The leukemia cells with FLT3-ITD plus TKD mutations express high levels of E-selectin ligands and CXCR4, ~5.6- and 10-fold increases compared to their parental FLT3-ITD mutated cells, respectively.

Blockade of E-selectin/CXCR4 with GMI-1359 reduces leukemia cell adhesion, migration, and homing to components of the BM niche in vitro and in vivo; and increases cell motility in the vascular niche in vivo.

Co-targeting E-selectin/CXCR4 with GMI-1359 and FLT3 with sorafenib markedly decreased leukemia cell engraftment in PB, reduced leukemia cell infiltration in the BM, liver, lung, and spleen, and extended overall survival in a FLT3-ITD-mutated AML PDX mouse model, which was resistant to 6 different treatment regimen, including two with Sorafenib.

Combination therapy with GMI-1359 and sorafenib enhanced normal hematopoiesis in mouse BM by upregulating hematopoiesis-related cytokines and chemokines, and increasing the numbers of megakaryocytes (16.5 fold) and myelocytes (4.5 fold) compared to controls.

Our findings suggest that co-targeting E-selectin, CXCR4, and FLT3 reduces leukemia burden and may protect normal hematopoiesis.

CONCLUSIONS
ACKNOWLEDGEMENTS

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