Combined Targeting of E-selectin/CXCR4 and FLT3 by GMI-1359 and Sorafenib Effectively Reduces Leukemia Cell Burden and Protects Normal Hematopoiesis in a Patient-derived AML Xenograft Model

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Background

Acute myelogenous leukemia (AML) is a hematological malignancy characterized by the accumulation of abnormal immature white blood cells. Internal tandem duplications in the FMS-like tyrosine kinase 3 (FLT3-ITD) account for about 30% of adult AML cases and confer poor prognosis (Kottaridis et al., 2003; Thiede et al., 2002). FLT3 inhibitors like sorafenib efficiently eliminate leukemia blast in the peripheral blood (PB), but frequently not in the bone marrow (BM) (Zhang et al., 2008). This suggests a protective effect of the BM on leukemia stem cells, which is mediated by E-selectin and CXCL12 on endothelial cells (ECs) and mesenchymal stem cells (MSCs) in the BM vascular and stromal niches (Horacek et al., 2013; Peled and Tavor, 2013). Our previous study demonstrated that targeting E-selectin and CXCR4 with the dual E-selectin/CXCR4 antagonist GMI-1359 markedly reduced leukemia cell adhesion to ECs and MSCs and eliminated the BM-mediated protection of leukemia cells during FLT3-targeted therapy in vitro, and effectively reduced leukemia cells in the BM in vivo (Zhang et al., 2016). Further, GMI-1359 combined with cytarabine and daunorubicin provided a pronounced survival benefit in FLT3-mutated leukemia mice, M4/11-bearing mice (Zhang et al., 2015).

Hypothesis

Combination targeting of CXCR4, E-selectin, and FLT3 could be effective in leukemia blast mobilization and increase killing by abrogating BM-mediated protection.

- Could enhance maintenance of normal hematopoiesis in the BM.

Methods

- Expression levels of cell surface E-selectin ligand and CXCR4 were assessed by flow cytometry after staining with anti-E-selectin and anti-CXCR4 antibodies in FLT3-ITD+ AML cell lines.

- After pretreatment with E-selectin inhibitory GMI-1359 or dual E-selectin/CXCR4 inhibitory GMI-1359+32.9 for 1 hour, MOLM14 cells were seeded on precoated charcoal layers for 24 hours. The adhesion cells were collected by trypsin digestion and counted. The percentage reduction in cell adhesion was calculated by comparing MCD13 positive cells in the outer chamber by flow cytometry.

- MOLM14 cells were treated with sorafenib for 4 hours, pretreated with GMI-1359 or GMI-1359+32.9 for 3 hours. Apoptosis induction was determined with flow cytometry by measuring annexin V positivity after gating on the EC population.

- Cells from a FLT3-ITD-mutated AML patient samples were injected into irradiated NSG mice. The mice treated treatment with vehicle (PBS), 32.9 μM sorafenib, 225 μM GMI-1359 or 32.9 μM sorafenib+225 μM GMI-1359 were used for the experiment. Cell viability was assessed by the trypan blue exclusion test. Cell adhesion was assessed by flow cytometry measuring E-selectin and GRO-A receptor expression. Mouse survival was estimated by the Kaplan-Meier method with log-rank test.

- Cytoplasmic/nuclear levels were determined with a murine cytokine antibody array (Millipore, Billerica, MA) by testing GM1359 leukemia cells in a MouseCytokine array kit. (GM1359 + 32.9-IFN-β4H) in the presence of GMI-1359 or GMI-1359+32.9 for 4 hours. The experiment was performed in triplicate.

- To prepare for murine E-selectin and transmembrane CXCR4 (iCXCR4) targeting with murine E-selectin antibodies, targeting experiments were performed in primary mouse BM microvasculature with 20X magnification. Error bars present the standard deviation for the mean values from four independent experiments.

Results

Fig. 1. FLT3-mutated Leukemia Cells Express High Levels of E-selectin Ligands and CXCR4, which Increase during Hypoxia

- FLT3-mutated leukemia cells express high levels of E-selectin ligands and CXCR4 which increase during hypoxia.

- FLT3-mutated leukemia cell adhesion to BM niche components, and enhances sorafenib-induced apoptosis.

Conclusions

- Blockade of E-selectin/CXCR4 with GMI-1359 reduces leukemia cell adhesion and migration to BM niche components, and enhances sorafenib-induced pre-apoptotic effects in vitro.

- Co-targeting E-selectin/CXCR4 with GMI-1359 and FLT3 with sorafenib markedly decreases leukemia cell engraftment in PB, reduced leukemia cell infiltration in liver, lung, and spleen, and extended survival in an AML PDX mouse model harboring FLT3-ITD mutations.

- Combination therapy with GMI-1359 and sorafenib enhanced normal hematopoiesis in mouse BM by upregulating hematopoiesis-related cytokines/chemokines and increasing the numbers of megakaryocytes and myelocytes.

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

Potential Conflict of interest: W. Fogler and J. Magnani are employees of GlycoMimetics, Inc.