Targeting E-Selectin with GMI-1271 Overcomes Microenvironment-Mediated Resistance to Venetoclax / HMA Therapy

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Introduction

Acute myeloid leukemia (AML) is an aggressive heterogeneous hematologic disease with high mortality in patients older than 60 years. Clinical studies have proven that combinations of FDA-approved Bcl-2 inhibitor, venetoclax and hypomethylating agents (HMA) are highly effective in elderly patients with AML (DiNardo et al., 2019). Despite improved remission rates, the duration of response is still short. Adhesion to the bone marrow (BM) niche is critical for AML initiation, progression and leukemic stem cells (LSC) survival after induction therapy. The vascular adhesion molecule, E-selectin (E-sel) has crucial roles in BM homing and engraftment in leukemia (Krause et al, 2006). Our hypothesis is that targeting the vascular niche in the BM microenvironment by inhibition of E-sel with GMI-1271 can further improve the venetoclax/HMA therapy for patients with AML. Here, we analyzed the underlying mechanisms of E-sel in AML and AML BM niches.

Models

In vitro

Human AML cells lines

Human umbilical vein endothelial cells (HUVEC)

Human healthy donor derived-mesenchymal stromal cells (MSC)

In vivo

AML-PDX derived from a venetoclax/HMA resistant AML patient harboring FLT3-ITD, NRAS, and GATA2 mutations.

Results

AML upregulates E-selectin expression in EC | **AML cell lines have different E-selectin binding potential**

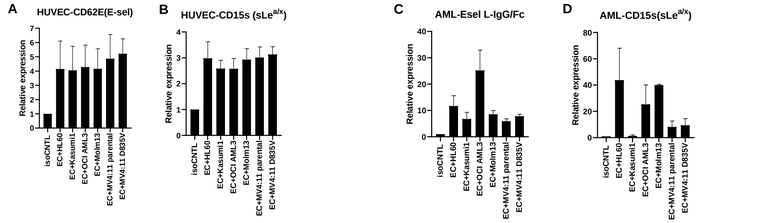

Fig 1. AML induces upregulation of E-selectin (A) and CD15-sLe$^{a/x}$ (B) in human endothelial cells (HUVEC). (C,D) E-selectin ligands and CD15s are differentially expressed in AML cell lines. Increased expressions of E-selectin Ligand (C) and CD15-sLe$^{a/x}$ (D) in AML after coculture with HUVEC.

Results

E-Selectin binding reduces proliferation and increases AML cell dormancy

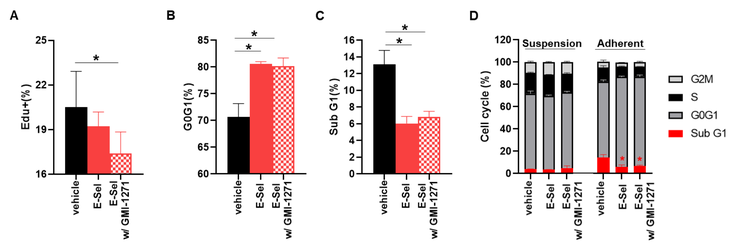

Fig 2. Changes of AML cell cycle status. (A) E-selectin reduces AML cell proliferation. Membrane bound E-selectin increases G0G1 cell cycle (B) but decreases sub-G1 phase in AML (C and D). * p <0.05 *vs.* vehicle CNTL (BSA).

E-selectin modulates CDK4/6 expression in AML | **E-selectin upregulates eNOS activation in EC**

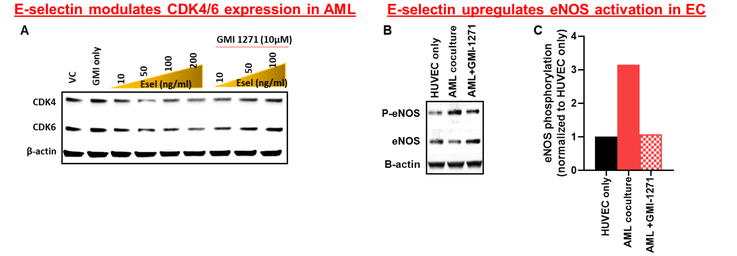

Fig 3. Representative images of western blot analysis. (A) CDK4/6 protein expressions in AML. (B and C) AML coculture induces eNOS activation and GMI-1271 restores phosphorylation of eNOS in EC.

CD44 surface expression in MSC is induced by soluble E-selectin binding

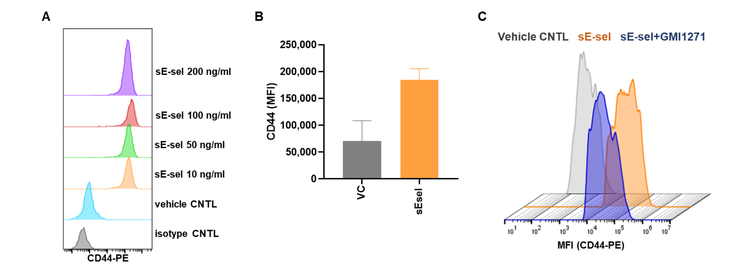

Fig 4. Normal human MSC was exposed to soluble E-selectin for 24 hours with or without GMI-1271. CD44 expression was measured by flow cytometry analysis (A and B). (C) Abrogated sE-selectin binding by GMI-1271 diminishes CD44 expression in normal human MSC.

Results

Combination of GMI-1271 and Venetoclax/HMA Induces significantly prolonged survival of AML-PDX from a patient who developed resistance to Venetoclax/HMA therapy

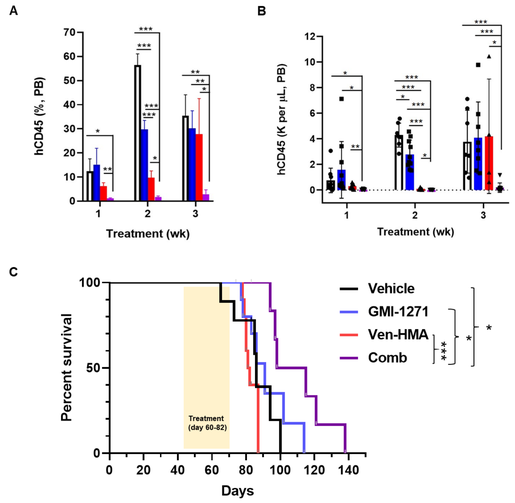

Fig 5. Percent (A) and absolute number (B) of human CD45$^+$ cells in peripheral blood circulation during 3 weeks of therapeutic agent(s) treatment. Once AML engraftment, mice were divided into 4 groups for treatment (40 mg/kg of GMI-1271;blue, 50 mg/kg of venetoclax + 5.5 mg/kg 5-Azacitidine;red) including vehicle control (open bars) and combination (purple bars). (C) Kaplan-Meier survival curves of AML-PDX.PDX cells from AML patient (ITD, NRAS, and GATA2 mutations, venetoclax+HMA resistant) were transplanted via tail vein injection into NSG mice. Drug treatment was performed from day 60 to 82 post-transplantation. *p< 0.05; **p<0.01; ***p<0.001.

Conclusions

- AML upregulates expressions of E-sel and its active binding molecule, CD15s in EC.
- E-sel binding reduces CDK4/6 expression in AML resulting in diminished AML proliferation and augmented G0G1 cell cycle arrest.
- E-sel modulates BM niche component cells: eNOS in EC and CD44 in MSC.
- Targeting E-sel mobilizes human AML cells and sensitizes them to venetoclax/HMA in a PDX model derived from a patient who had developed resistance to venetoclax/HMA.