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Background

Aberrant activation of the FMS-like tyrosine kinase-3 (FLT3) can be driven by internal tandem duplication (ITD) mutations in the FLT3 gene, and is commonly observed in patients with acute myeloid leukemia (AML). Hence, FLT3 represents an attractive therapeutic target in AML (Weisberg et al., 2000). Indeed, several small molecule FLT3 inhibitors including sorafenib have showed encouraging efficacy in reducing leukemia blasts in the peripheral blood in FLT3-mutated AML patients. However, these agents have little effect on leukemic stem cells residing in the bone marrow (BM) microenvironment (Borthakur et al., 2011; Fathi and Chabner, 2011; Zhang et al., 2005). Furthermore, the BM microenvironment is enriched with cytokines and adhesion molecules, such as CXCR4 and E-selectin, which are believed to provide AML cells protection against chemotherapy agents (Horacek et al., 2013; Peled and Tavor, 2013). In fact, treatment with sorafenib markedly upregulated CXCR4 levels in FLT3-mutated AML cells. In addition, leukemia cells can activate endothelial cells (ECs) and stromal cell (mesenchymal stromal cells, MSCs) that induce adhesion of a sub-set of the leukemia cells through E-selectin. The adherent AML cells are then sequestered in a non-proliferative state that further protects them from chemotherapy (Pozzobon et al., 2013). Therefore, blocking CXCR4 and E-selectin in parallel could theoretically eliminate the protection provided by the interaction of leukemic cells in their BM microenvironment and enhance effectiveness of chemotherapy in FLT3-mutant AML patients.

Materials and Methods

Design: E-selectin/CXCR4 dual inhibitor GM1-1359 and E-selectin inhibitor GM-1271 were provided by Stryphlnetica, Inc. (Rockville, MD; CXCR4 antagonist, platelet and the FLT3 Cell line: The ITD mutated AML cell line MOLM14, MOLM-11, MV-4-11; and murine FLT3 ITD+Tbx20CreER Tg mice with FLT3 drive in BM that were injected with 106 Tbx20CreER+ cells were used (Wheeler et al., 2011) to test cell lines and L-gasim. Human mesenchymal stem cells (MSCs), endothelial cells HUVEC and murine MSCs (MSCs) were cultured under standard culture conditions. Flow Cytometry: Cell surface antigens were stained with different conjugated-specific antibodies and measured by a FACScan flow cytometer (BD Biosciences, San Jose, CA). Mean fluorescence intensity (MFI) was analyzed using FlowJo (Tree). Cell proliferation was determined by flow-cytometric measurement of Annexin-V/FLUOS and propidium iodide staining. Adhesion Assays: MSCs and HUVEC/TeloHAECs cells were pre-seeded to 24-well plates for 24 hours of incubation for feeder layer formation. The feeder layer cells and leukemia cells were preincubated with compounds for 1 hour. For treatment, the leukemia cells were cultured with the feeder layer for an additional 24 hours. Alternatively, the suspension cells were treated with compounds for 24 hours after gaining the human CD45 positive population. Animal Study: Female SCID beige mice (6-7 weeks) were injected with MV-4-11 (Tbx20CreER+ITD) cells. A steady state of 50,000 FLT3+ cells was maintained for 1 week. FLT3+ cells were harvested by flushing the bone marrows (BM) of mice with PBS and mixed with 20% fetal bovine serum (FBS). All the cell suspensions were then plated in 6-well plates. Colony formation assay: Cells were cultured in suspension for two weeks to allow colony formation. Cells were then washed, trypsinized, and harvested. Colony formation was measured by colony counting. The experiment was repeated at least three times.

Results

Hypoxia upregulates CXCR4, E-selectin, HECA452, and CD44 expression in FLT3-ITD Mutated Leukemia Cells.

GM1-1359 Effectively Reduces Adhesion and Migration of Leukemic Cell to MSCs and ECs (h) in a Co-culture System (a)

GM1-1359 Abrogates Microenvironmental Protection of Sorafenib-Induced Leukemic Cell Killing

Combination of GM1-1359 with C and D NDR Extends Survival of Leukemia-bearing Mice Engrafted with FLT3-ITD Mutated MV4-11 Cells

Conclusions

Most FLT3-ITD-mutated AML cell lines have high levels of CXCR4 expression, which further increases in hypoxia (i.e., 1% oxygen).

These FLT3-ITD leukemic cells also express high level of E-selectin, HECA452, and CD44.

GM1-1359 reduces leukemic cells adhesion and migration to MSCs and ECs.

GM1-1359 effectively abrogates protection provided by stromal/endothelial cells and/or hypoxia and enhances sorafenib-induced apoptosis in FLT3-ITD-mutated leukemic cells.

Combination of GM1-1359 with cytoreductive and duromobilin profoundly extends survival of MV4-11-engrafted mice.