GlycoMimetic Antagonist of E-selectin, GMI-1271, Enhances Therapeutic Activity of the Hypomethylating Agent, 5-Azacytidine, in the KG1 Model of AML
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Introduction

GMI-1271 disrupts the relationship between cancer cells and tumor microenvironment

E-selectin:
• Is constitutively expressed in the bone marrow microvasculature and levels are upregulated in AML.
• Binds with the E-selectin ligand expressed on AML cells.

GMI-1271 is a novel E-selectin antagonist that when used in combination with chemotherapy results in improved survival in mouse syngeneic and xenograft AML tumor models. GMI-1271 in combination with two chemotherapeutic regimens in in early clinical trials for the treatment of AML. Azacitidine (5-AC) is a DNA-methyltransferase inhibiting cytidine nucleoside analog that at low doses induces DNA hypomethylation and transcriptional activation, while at higher doses is directly cytotoxic to neoplastic cells including AML blasts. 5-AC is approved in Europe for the treatment of limited populations with AML. We evaluated GMI-1271 in combination with 5-AC in the KG1 AML tumor model to assess the potential for therapeutic benefit of the combination.

NIH mice (10/group) received iv injections of KG1 cells, and were treated with saline, GMI-1271 alone, 5-AC alone, or the combination of GMI-1271 and 5-AC. The median survival time (MST) of mice treated with 5-AC was 88 days and statistically different (P=0.002) to groups treated with saline (MST=69.5 days) or GMI-1271 alone (MST=69 days). All mice treated with saline or GMI-1271 alone succumbed to progressive tumor growth. At study conclusion (Day 104 post tumor injection) 20% of mice treated with 5-AC remained alive. Importantly, the therapeutic activity of 5-AC was significantly enhanced when combined with GMI-1271 (MST=104 days, P=0.0140 compared to 5-AC alone) with 70% of mice surviving to study conclusion. These results indicate that 5-AC and GMI-1271 AML blast interaction in the KG1 model promotes the anti-tumor activity of 5-AC and that GMI-1271 attenuates this progression.

To investigate the nature of the observed in vivo activity of GMI-1271 and 5-AC, KG1 cells were cultured for 96h in a noncytotoxic concentration (100 nM) of 5-AC and the reactivity of the cells to HECA-452 (an antibody that recognizes an E-selectin carbohydrate ligand) and binding to E-selectin were determined by flow cytometry. Treatment with 5-AC resulted in a 28% increase in reactivity of cells to HECA-452 and a 35% increase in binding to E-selectin. Further in vitro assays of selectin function revealed an increase in the adhesion of 5-AC-treated KG1 cells to E-selectin. Notably, the enhanced adhesion of KG1-1 cells to E-selectin was reversed using GMI-1271. Collectively, these results demonstrate that 5-AC can lead to increased expression of E-selectin ligands on AML cells and that the therapeutic potential of 5-AC could be improved by combination with GMI-1271.

Results

Figure 1. Activity of 5-Azacitidine Alone or in Combination with GMI-1271 in the KG1 AML Model

Formalin-fixed pieces of KG1 tumor tissue were stained with KG1 and KG1/5-AC using an immunoperoxidase method. 5-Azacytidine, in the KG1 Model of AML

Figure 2. Cell viability assay: 5-Azacitidine and decitabine treatment of AML cell lines

The cells of a 96-well plate were seeded with 1x10^5 KG1 or KG1/5-AC human AML cells. A 10-fold serial dilution of 5-azacitidine was prepared in R10 media 1:2 and added to appropriate wells. Other works of 5-Azacitidine in AML cell lines in vitro IC50 of 5-Azacitidine in KG1 and KG1/5-AC has been shown.

Figure 3. Incubation of AML cell lines with non-cytotoxic concentrations of 5-Azacitidine or decitabine increases reactivity with HECA-452 and E-selectin

Cells were subcultured into 104 10-ml tubes. Cells were treated at 5-AC (top) or 0.1% DMSO (control). The reactivity of the cells with the HECA-452 monoclonal antibody, which specifically reacts with GalNAc-XylXyl carbohydrate antigen (CLA), a carbohydrate domain shared by sialyl Lewis X and sialyl Lewis A antigens and is a surrogate marker of E-selectin ligand, was determined by flow cytometry. In addition, the binding of the E-selectin-Fc chimera conjugated with IR-phycoerythrin was measured by flow cytometry.

Figure 4. The effect of 5-azacitidine treatment of AML cells on adhesion to E-selectin

Cells were treated daily with 100 nM freshly prepared 5-azacitidine for 4 days then labeled with the fluorescent, carboxyfluorescein diacetate Calcein AM. The cells were added to a 96-well plate that had previously been coated with 7 µg/ml E-selectin, and the cell adhesion was allowed to stabilize for 45 minutes at room temperature. The plate was washed to remove unbound cells, and the adherent cells were measured by flow cytometry.

Conclusions

• These findings suggest that hypomethylating agents increase the adhesion of leukemic blasts in the bone marrow via modulation of E-selectin ligand expression and therefore potentially hinder the intended anti-leukemic effect. This could explain a source of chemoresistance and potential for relapse.
• The addition of GMI-1271 post-binding of KG1 cells to E-selectin led to an approximate 90% uncoupling of adhesion, demonstrating that the effect could be reversed with the E-selectin antagonist.
• The significance of these data was demonstrated in the KG1 AML tumor model. Further investigation of GMI-1271 in combination with 5-azacitidine lead to a statistically significant increase in MST and survival compared to 5-azacitidine alone.