Abstract # 2867

Glycomimetic Antagonist of E-selectin, GMI-1271, Enhances Therapeutic Activity of the Hypomethylating Agent, 5-Azacitidine, in the KG1 Model of AML

Theodore A.G. Smith, William E. Fogler, Ji-Won Lee, John L. Magnani
GlycoMimetics, Inc., Rockville, MD

Abstract

Binding of AML blasts to E-selectin activates cellular survival pathways leading to chemoresistance. GMI-1271 is a novel E-selectin antagonist that when used in combination with chemotherapy results in improved survival in mouse syngeneic and xenogeneic AML tumor models. GMI-1271 in combination with two chemotherapy regimens is 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.

NSG mice (10/group) received i.v. 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 E-selectin/AML blast interaction in the KG1 model protects from the anti-tumor activity of 5-AC and that GMI-1271 attenuates this protection.

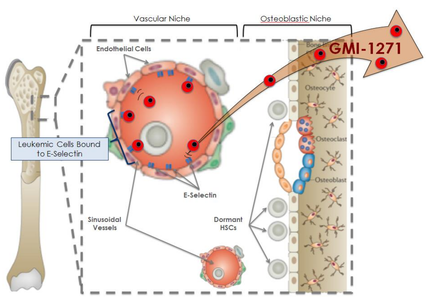
To investigate the nature of the observed in vivo activity of GMI-1271 and 5-AC, KG1 cells were cultured for 96 h in a noncytotoxic concentration (100 nM) of 5-AC and the reactivity of the cells to HECA-452 (an antibody that recognizes an E-selectin carbohydrate ligand) and binding to E-selectin were determined by flow cytometry. Treatment with 5-AC resulted in a 28% increase in reactivity of cells to HECA-452 and a 32% increase in binding to E-selectin. Further in vitro assays of static adhesion revealed an increase in the adhesion of 5-AC-treated KG1 cells to E-selectin. Notably, the enhanced adhesion of KG-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.

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.



In preclinical models

- Prevents trafficking of tumor cells to the bone marrow
- Disrupts cell adhesion-mediated drug resistance (CAMDR) within bone marrow microenvironment
- Inhibits activation of cancer survival pathways (e.g. Nf-Kb)
- Protects normal HSCs by enhancing quiescence and ability for self-renewal
- Reduces chemotherapy-associated toxicity (e.g. mucositis)

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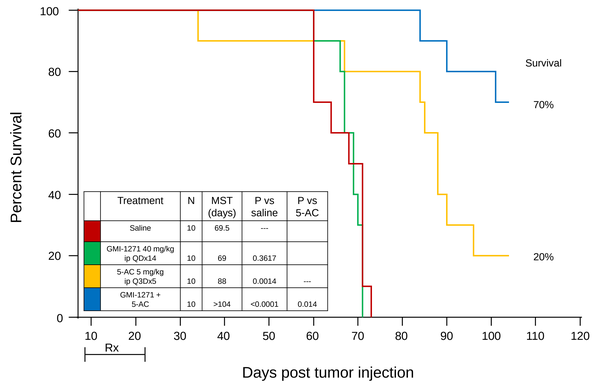
Results

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

Female NSG mice (6 wks of age) were injected iv with KG-1 human AML tumor cells modified with mCherry and luciferase ($5.0x10^6$ cells/mouse). Beginning 7 days post injection, mice were randomized into 4 cohorts and treated with: Cohort 1- saline (0.2 mL/20g ip qdx14); Cohort 2- GMI-1271 (40 mg/kg ip qdx14); Cohort 3- 5-azacitidine (5 mg/kg ip q3dx5); and Cohort 4- the combination of GMI-1271 and 5-azacitidine. The efficacy of the treatment on survival was estimated by the Kaplan-Meier method, and log-rank statistics was used to test for differences in survival.



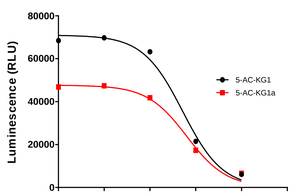
Treatment	N	MST (days)	P vs saline	P vs 5-AC
Saline	10	69.5	---	
GMI-1271 40 mg/kg ip QDx14	10	69	0.3617	
5-AC 5 mg/kg ip QDx5	10	88	0.0014	---
GMI-1271 + 5-AC	10	>104	<0.0001	0.014

Summary

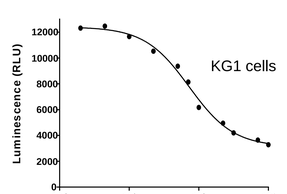
- **Treatment of mice with GMI-1271 alone or together with 5-azacitidine was well tolerated.**
- **Median survival times (MST) of mice treated with saline or GMI-1271 was 69.5 and 69 days respectively.**
- **Treatment of mice with 5-azacitidine led to an increase in life span (MST=88 days, p<0.002 vs. saline).**
- **The combination of GMI-1271 and 5-azacitidine led to a further therapeutic enhancement over that obtained with 5-AC alone (MST>104, p=0.014 vs. 5-azacitidine).**

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

The wells of a 96-well plate were seeded with $1x10^5$ KG1 or KG1a human AML cells. A 10-fold serial dilution of 5-azacitidine was prepared in PBS pH 7.2 and added to appropriate wells. Other wells of KG1 cells were treated with various concentrations of decitabine. The plates were incubated at 37ºC in 5% CO_2 for 96h. Fresh 5-azacitidine and decitabine were added daily. Cell viability was determined using the CellTiter-Glo Luminescent Cell Viability Assay (Promega).



	5-AC-KG1	5-AC-KG1a
EC50	518.9	637.2



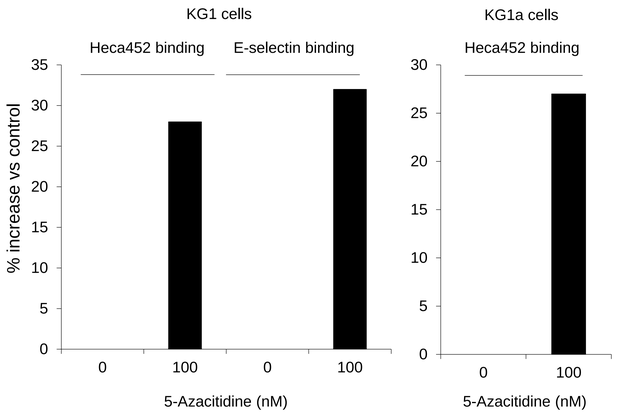
EC50	53.00

Summary.

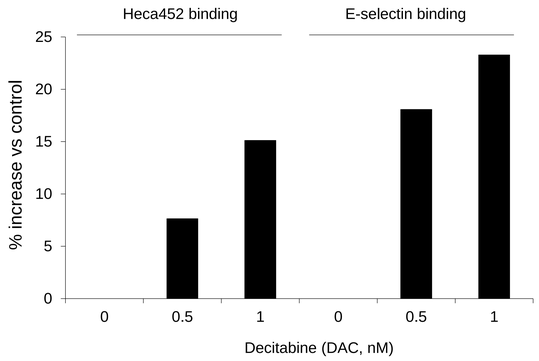
- **The EC_{50} of 5-azacitidine was 518 nM and 637 nM on KG1 and KG1a cells, respectively.**
- **The EC50 of decitabine on KG1 cells was 53 nM.**
- **At 100 nM 5-azacytidine and 1 nM decitabine, there was little or no cytotoxicity. These concentrations were used for further experiments to examine the effect of 5-azacitidine or decitabine on expression of E-selectin ligands.**

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 cultured for 96h in 100 nM 5-azacitidine (top) or 0.5 or 1 nM decitabine (bottom). The reactivity of the cells with the HECA-452 monoclonal antibody, which specifically reacts with Cutaneous Lymphocyte 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 E-selectin-PE (E-selectin-Fc chimera conjugated with R-phycoerythrin) was measured by flow cytometry.



KG1 cells

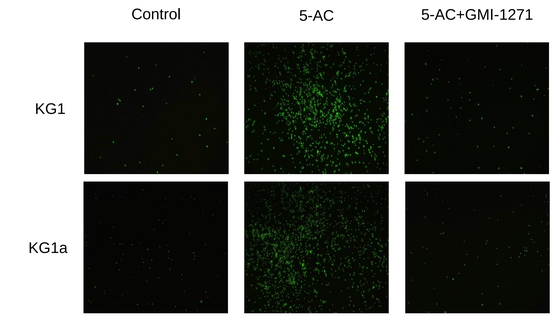


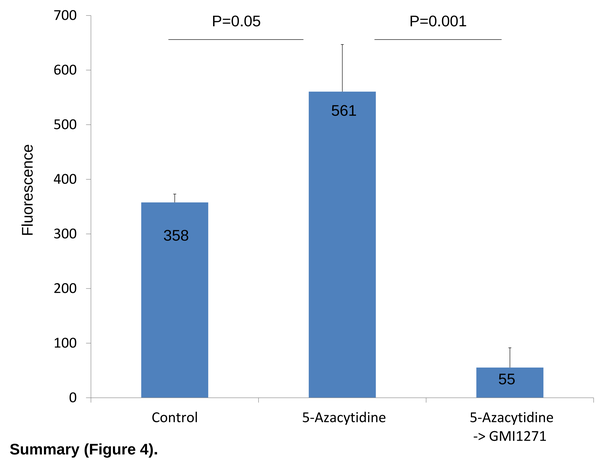
Summary (Figure 3).

- **These results demonstrate that 5-azacitidine or decitabine led to an increased expression of E-selectin ligands on AML cells, whether measured by reactivity with HECA-452 or by binding of E-selectin.**

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, cell-permeant dye Calcein AM. The cells were added to a 96-well plate that had previously been coated with 2 μg/mL E-selectin-Fc and the cells were allowed to adhere for 45 minutes at room temperature. The wells were washed 3 times with HBSS and cell adhesion was assessed by fluorescence microscopy and by measuring fluorescence using a FlexStation3 plate reader (Molecular Probes, Ex 485 nM, Em 538 nM, cutoff 530 nM). To determine if the E-selectin antagonist GMI-1271 could release cells from previous adhesion to E-selectin, wells were further incubated with 100 μM GMI-1271 for 30 minutes at room temperature.





Summary (Figure 4).

- **Incubation of AML cells with 5-azacitidine significantly enhanced adhesion to E-selectin (p=0.05).**
- **GMI-1271 treatment of previously attached cells significantly led to cellular release (p=0.001).**

Conclusions

- These findings suggest that hypomethlyating 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 where treatment of mice with GMI-1271 in combination with 5-azacitidine lead to a statistically significant increase in MST and survival when compared to 5-azacitidine alone.