Abstract # 2826

ADMINISTRATION OF THE DUAL E-SELECTIN/CXCR4 ANTAGONIST, GMI-1359, RESULTS IN A UNIQUE PROFILE OF TUMOR MOBILIZATION FROM THE BONE MARROW AND FACILITATION OF CHEMOTHERAPY IN A MURINE MODEL OF FLT3 ITD AML

William E. Fogler, Henry Flanner, Curt Wolfgang, Jeff Smith, Helen Thackray, and John L. Magnani
GlycoMimetics, Inc., Rockville, MD, USA

Background

A drawback of the current standard of care with chemotherapeutic agents is that these drugs tend to be ineffective at accessing and killing cancerous cells in the bone marrow, thereby reducing the treatment's overall potency. Recently, E-selectin and the CXCR4 ligand, CXCL12 have been shown to be constitutively expressed in vascular niches in the bone marrow where they co-localize and bind with leukemic cells thereby protecting these cells from the effects of chemotherapy drugs. With the limited penetration of chemotherapeutic agents into the bone marrow, the ability of E-selectin ligands and CXCR4 to retain malignant cells within the bone marrow may increase the potential for poor clinical response or relapse in some patients with hematological malignancy. Disruption of malignant cellular interactions with either E-selectin or CXCR4 would disrupt bone marrow adhesion and retention, and mobilize cancer cells out of the bone marrow into the blood stream, making them more susceptible to cytotoxic chemotherapy.

Introduction

GMI-1359 is a rationally-designed parenteral small molecule glycomimetic (MW = 1115) with dual antagonistic activity to ligands for E-selectin and CXCR4. We recently reported that targeting CXCR4/E-selectin with GMI-1359 showed efficient mobilization of AML blasts in to the circulation, and significant prolonged survival of mice in a *FLT3*-ITD mutant AML xenograft *(1, 2)*. Here we report a novel profile of leukemic cell mobilization induced by GMI-1359, in comparison to either CXCR4 or E-selectin antagonism alone, its impact on survival when combined with chemotherapy, and toxicity profile supporting entry to the clinic.

Results

Table 1. Competitive Binding Activity (IC50) of GMI-1359 Against E-selectin and CXCR4

GMI-1359 was assessed for inhibition of sialyl LeX binding to immobilized E-selectin and α-CXCR4 antibody binding to Jurkat cells. IC50's (μM) were determined.

Compound	E-selectin	CXCR4
GMI-1359	1.05	~1.0

Summary
- The small molecule glycomimetic, GMI-1359, inhibits ligand binding to both E-selectin and CXCR4 with approximate equal potencies

Table 2. Comparative activity of GMI-1359 and AMD-3100 in the Cerep SafetyScreen 87

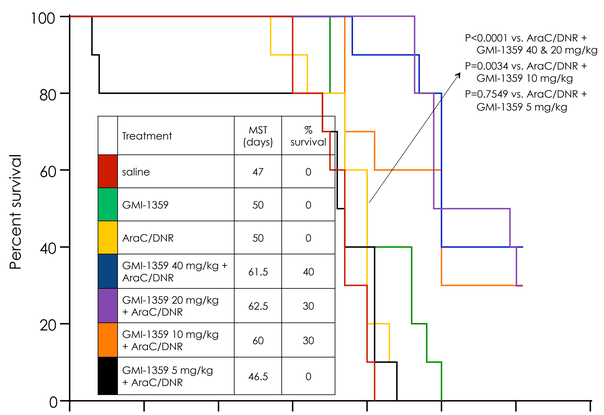
The inhibitory/stimulatory activity of 10 μM GMI-1359 and 10 μM AMD-3100 was evaluated in enzyme and radioligand binding assays. Significant responses were defined by >50% inhibition/stimulation.

Target	AMD-3100	GMI-1359
Adrenergic α_{1B}	77	3
Adrenergic α_{2A}	64	12
Calcium Channel N-Type	102	1
Glutamate, NMDA, polyamine	61	8
Muscarinic M_1	86	16
Muscarinic M_2	77	30

Summary
- No significant activity of GMI-1359 for 87 primary molecular targets (13 enzyme and 74 binding assays), including H1. In contrast AMD-3100 was noted to have significant responses in 6 assays, as previously documented and summarized above.

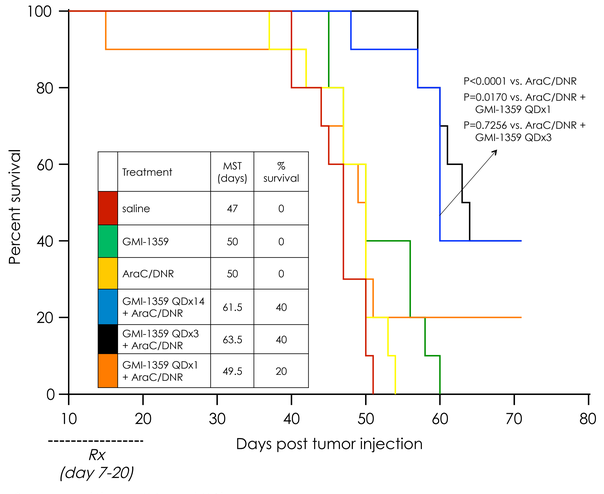
Figure 1A. Efficacy **of GMI-1359 in Combination with AraC/DNR in MV4.11 Tumor Bearing NCR Mice – Dose Response**

Female NCR mice (6-7 wks of age) were injected iv with MV4-11 FLT3 ITD human AML tumor cells modified with mCherry and luciferase (5.0 x10^6 cells/mouse), and beginning 7 days post injection, received saline alone qdx14, GMI-1359 (40 mg/kg ip qdx14), cytarabine (AraC, 300 mg/kg, ip) + daunorubicin (DNR, 3 mg/kg, iv), or a combination of GMI-1359 (40, 20, 10 and 5 mg/kg ip qdx14) and AraC/DNR (Figure XA). Alternatively, mice received saline alone qdx14, GMI-1359 (40 mg/kg ip qdx14), AraC/DNR as described above, or a combination of AraC/DNR and GMI-1359 (40 mg/kg ip qdx14, 40 mg/kg ip qdx3, 40 mg/kg ip qdx1). 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	MST (days)	% survival
saline	47	0
GMI-1359	50	0
AraC/DNR	50	0
GMI-1359 40 mg/kg + AraC/DNR	61.5	40
GMI-1359 20 mg/kg + AraC/DNR	62.5	30
GMI-1359 10 mg/kg + AraC/DNR	60	30
GMI-1359 5 mg/kg + AraC/DNR	46.5	0

Figure 1B. **Efficacy of GMI-1359 in Combination with AraC/DNR in MV4.11 Tumor Bearing NCR Mice – Impact of Interval Dosing**

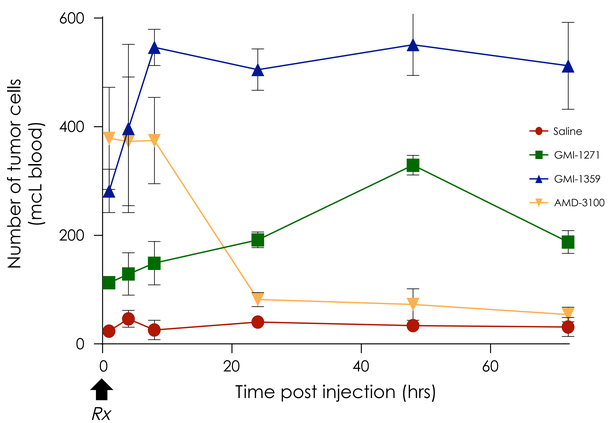


Treatment	MST (days)	% survival
saline	47	0
GMI-1359	50	0
AraC/DNR	50	0
GMI-1359 QDx14 + AraC/DNR	61.5	40
GMI-1359 QDx3 + AraC/DNR	63.5	40
GMI-1359 QDx1 + AraC/DNR	49.5	20

Summary (Figure XA and XB)
- Treatment of mice with GMI-1359 alone or together with AraC/DNR was well-tolerated
- Median survival times (MST) of mice treated with saline, GMI-1359 or AraC/DNR was 47, 50 and 50 days, respectively; all mice succumbed to progressive disease by study conclusion (Day 71)
- Daily administration of GMI-1359 given for 14 days demonstrated dose-dependent anti-tumor activity when combined with chemotherapy
- The enhanced anti-tumor effects of the combination could be achieved with GMI-1359 dosing only during the chemotherapeutic regimen (3 days)

Figure 2. Mobilization Kinetics of MV4.11 AML Cells from the Bone Marrow Following a Single IP Administration of GMI-1359, E-selectin antagonist or AMD-3100

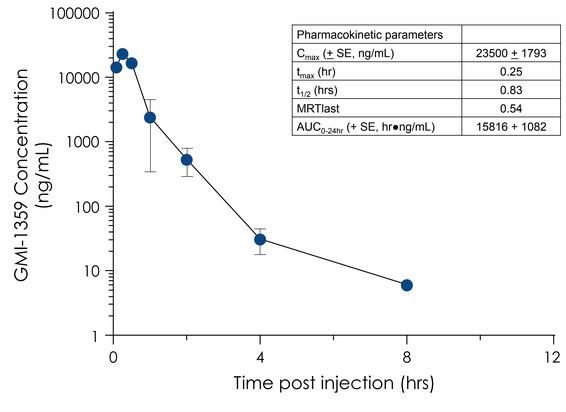
A comparison of tumor mobilization by GMI-1359 to E-selectin or CXCR4 antagonists was determined in NCR mice with established BM infiltrative MV4-11 FLT3 ITD human AML tumor (modified with mCherry and luciferase). Mice (n=18/group) were injected with GMI-1359 (40 mg/kg), a potent E-selectin antagonist (40 mg/kg), or plerixafor (5 mg/kg) and blood samples were assessed by flow cytometry at multiple times for human AML cells (mCherry) and WBC (murine CD45).



Summary
- Appearance of human AML cells in peripheral blood increased ~16-fold compared to untreated mice 8 h post treatment with GMI-1359, and remained elevated for the duration of the study (72 h)
- Tumor cell mobilization following treatment with the E-selectin antagonist was gradual, reaching a 9.7-fold increase 48 h post injection and trending down at 72 h
- Administration of AMD-3100 induced a rapid 11-fold mobilization of tumor cells by 1 h returning to baseline at 24 h
- At the 24, 48 and 72 h time points, murine WBC count did not change in any group.

Figure 3. Average Plasma Concentration for GMI-1359 versus Time Following Intraperitoneal Administration in Mice at 10 mg/kg

The pharmacokinetics of GMI-1359 was evaluated in fasted male CD-1 mice. Dosing was performed in triplicate for each time point. Each mouse received a single bolus dose into the peritoneal cavity at time zero on the day of dosing. Blood samples were collected up to 24 hrs post dose in sodium citrate. Plasma levels of GMI-1359 was determined using a LC-MS/MS method and individual plasma concentration versus time data was subjected to noncompartmental analysis using WinNonlin. Plasma concentrations below the level of quantitation (5 ng/mL) were assigned a value of zero.



Pharmacokinetic parameters	
C_{max} (± SE, ng/mL)	23500 ± 1793
t_{max} (hr)	0.25
$t_{1/2}$ (hrs)	0.83
MRTlast	0.54
$AUC_{0\text{-}24hr}$ (+ SE, hr•ng/mL)	15816 + 1082

Table 3. Toxicokinetic Parameters at the NOAEL in Rat and Monkey after a 1 hour IV Infusion

The toxicity of GMI-1359 was evaluated in Wistar Han rats and Cynomolgus monkeys after daily administration via one hour IV infusions for 28 days followed by a 14 day recovery period.

Species	NOAEL (mg/kg)	Cmax (ng/mL)	AUC (hr•mg/mL)
Wistar Han Rat	10 male; 40 female	18100; 67900	19600; 71700
Cynomolgus Monkey	30	80,900	162,500

Summary
- The NOAEL in monkey was 40 mg/kg due to transient clinical observations with no significant pathological findings at doses up to 80 mg/kg
- The NOAEL in rat was 10 mg/kg for males and 40 mg/kg for females due to histologic findings in the kidney of male rats and clinical observations in female rats
- Clinical observations in rat and monkey were similar and comprised hypoactivity, tremors, partial closure of the eyes and dilated pupils
- Toxicokinetic evaluation in both species demonstrated dose related increases in exposure, no apparent difference between sexes, and no accumulation 28 days of daily administration
- GMI-1359 was tested for off-target interactions, mutagenicity, and clastogenicity with no significant findings observed
- DMPK studies indicated a low likelihood of significant liver metabolism, no interaction with P-gp as substrate or inhibitor, moderate binding to plasma proteins and good plasma stability
- Taken together the non-clinical toxicology and DMPK profile of GMI-1359 support entry into human clinical studies

Conclusion

- The profile of GMI-1359 induced tumor cell mobilization is distinct from both plerixafor (rapid mobilization of short duration) and an E-selectin antagonist (gradual mobilization of long duration)
- As E-selectin but not CXCR4 has been reported to be critical for tumor cell entry into the BM, the sustained presence of tumor cells in circulation after GMI-1359 treatment could be the result of blocking not only CXCR4 but also E-selectin, thereby inhibiting their re-entry into the BM
- This novel kinetic signature of mobilization by dual inhibition of two important adhesion molecules and accompanying improvement in survival over standard chemotherapy support the potential for combination with current treatments to improve outcomes for patients with marrow involved disease
- First-in-human trials have been initiated

References

1. Zhang et al., ASH Abstract #3790, 2015.
2. Zhang et al., AACR Abstract #3284, 2016.