Abstract # 5211

DUAL E-SELECTIN AND CXCR4 INHIBITION REDUCES TUMOR GROWTH AND METASTATIC PROGRESSION IN AN ORTHOTOPIC MODEL OF OSTEOSARCOMA

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Abstract

Osteosarcoma is the most common bone cancer in children and young adults and has a strong propensity to develop lung metastases. E-selectin is known to be involved in the focal adhesion of tumor cells to cytokine exposed endothelial cells and we postulated may play a central role in osteosarcoma progression. Previously we identified that SDF-1, the main ligand for CXCR4, was upregulated in the pre-metastatic niche (Kaplan et al Nature 2005). Many tumor cells express CXCR4 and may use this signaling pathway to direct disseminated tumor cells to pre- and early metastatic sites in the lung. Based on these findings we examined human osteosarcoma cell lines and primary patient derived xenografts (PDXs) for the expression of CXCR4 and E-selectin ligands by flow cytometry. We found robust expression of these ligands in the majority of both the human osteosarcoma cell lines and PDXs examined. We therefore, investigated the impact of targeting these two axes on metastatic progression of orthotopic osteosarcoma using a small molecule, glycomimetic compound with dual inhibitory activity against E-selectin and CXCR4, GMI-1359. Five days post paratibial injection the HOS cell line, female NMRI-nu mice (n=12/group) were treated with saline; GMI-1359 alone (40 mg/kg IP BID x 25 days); doxorubicin (DOX) alone (5 mg/kg IV days 5, 15 and 25), or the combination of GMI-1359 and DOX. All treatments were well tolerated. The % tumor volume in treatment/control on day 27 of mice treated with GMI-1359, DOX or the combination was 35.5, 36.7 and 32.5, respectively. At study conclusion the incidence of lung metastases was approximately 60% and 50% in mice treated with saline or DOX and 15% in mice treated with GMI-1359 alone or in combination with DOX. Moreover, the extent of ectopic bone formation and/or osteolytic lesions was lower in mice treated with GMI-1359 compared to saline and DOX. These results indicate that the E-selectin and CXCR4 axes are important for the progression of osteosarcoma, and further, that inhibition of these two pro-tumor growth components by GMI-1359 has a therapeutic advantage over chemotherapy alone. Furthermore, studies in the adjuvant setting can provide proof of concept of utility of targeting CXCR4 and E-selectin ligands in the metastatic niche as a therapeutic strategy to limit metastatic progression in high risk patients.

Background

- GMI-1359, a small molecule glycomimetic, inhibits ligand binding to E-selectin and CXCR4.
- Previous studies showed that GMI-1359 reduced tumor growth and osseous alterations in a prostate cancer model of bone metastases (Gravina et al., AACR 2015).
- Determinants of osteosarcoma progression include E-selectin and stromal cell-derived factor-1α that interact with E-selectin ligands and CXCR4, respectively.
- These molecular interactions may be targeted for pharmacologic treatments of osteosarcoma.

Results

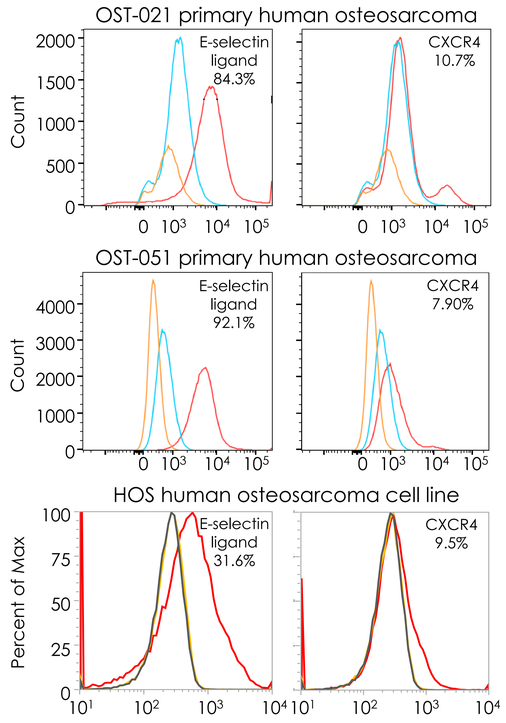
Table 1. Binding of GMI-1359 against E-selectin or CXCR4

GMI-1359 (dual E-selectin/CXCR4 antagonist) was assessed for inhibition of (1) sialyl LeX binding to immobilized E-selectin and (2) anti-CXCR4 binding to Raji cells. IC50's (μM) were determined and summarized in Table 1.

Compound	E-selectin	CXCR4
GMI-1359	1.0	0.5

Figure 1. Detection of E-selectin ligands and CXCR4 on Patient-derived Osteosarcoma Cells and the HOS cell line

Primary human osteosarcoma cells or the human osteosarcoma cell line HOS were incubated with either a phycoerythrin-labeled E-selectin Ig chimeric molecule or anti-CXCR4 (clone 12G5) and the percentage of reactive cells was determined by flow cytometry. Controls included unlabeled cells or nonspecific IgG2a.



Summary (Table 1 and Figure 1).

- The small molecule glycomimetic, GMI-1359, inhibits ligand binding to both E-selectin and CXCR4.
- Human primary osteosarcoma and the osteosarcoma cell line HOS, express E-selectin ligands and CXCR4.
- GMI-1359 was subsequently evaluated for anti-tumor activity in the E-selectin ligand and CXCR4 positive, HOS osteosarcoma cell line.

Table 2. Study Protocol to Assess Anti-tumor Activity of GMI-1359 alone and in Combination with Doxorubicin (DOX) in the HOS Tumor Model

Treatment Group	Dose (mg/kg)	Regimen	Study Parameters
Vehicle	--	IP, bid x 25 days	Body weight, tumor burden, radiography, histology
GMI-1359	40	IP, bid x 25 days	
DOX	5	IV, day 5, 15 and 25	
GMI-1359 + DOX	40 5	as above	

Figure 2. GMI-1359 Inhibits Tumor Progression of Human Osteosarcoma in the HOS Paratibial Model

Female NMRI-nu mice (6 wks) received paratibial injections with HOS human osteosarcoma (2 x 10^6/mouse). Beginning 5 days post injection, mice were randomized into 4 groups (n=12 mice/group) and treated as described in Table 2. Tumor volumes were measured twice/week from day 0 to day 27. The mean tumor burden (±SD) was calculated per group and % T/C determined.

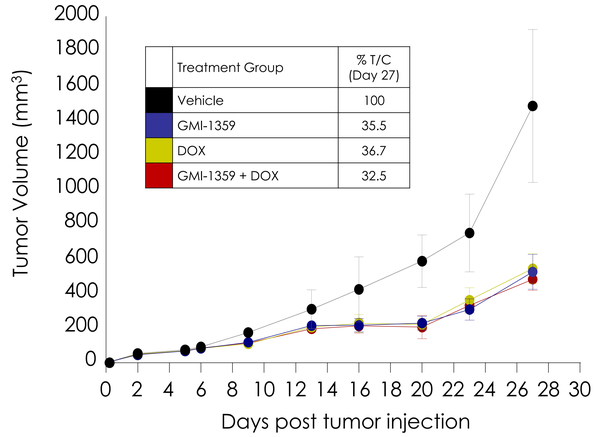


Figure 3. GMI-1359 Inhibits the Development of Pulmonary Metastases from Primary Osteosarcoma Lesions

At study termination (Day 30), mice were necropsied and the presence or absence of macroscopic pulmonary metastases was determined .

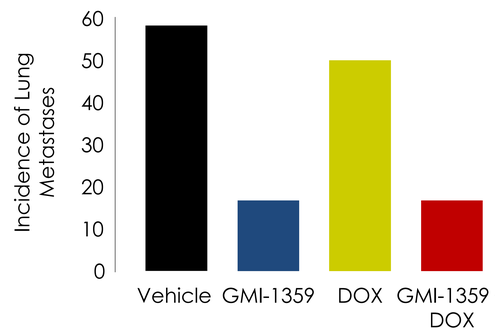
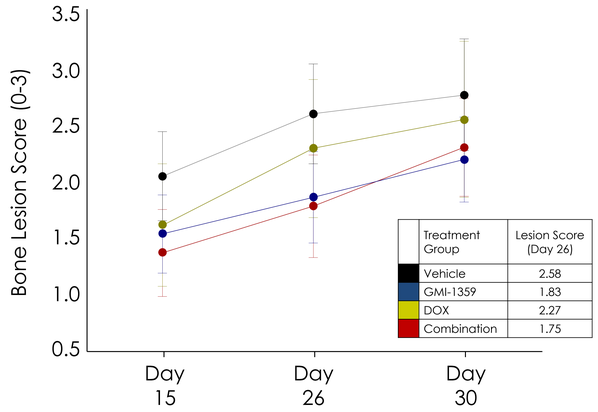


Figure 4. GMI-1359 Attenuates Osteosarcoma-induced Osseous Pathology

The effects of GMI-1359 and doxorubicin on HOS-induced bone pathology was monitored by radiography of the right tibia at study days 15, 26 and 30 (study termination). Images were blinded and scored for extent of ectopic bone formation (0-3) and osteolysis (0-3). Mean lesion scores (± SD) were determined for each group.



Summary (Figures 2-4).

- All treatments were well tolerated by tumor bearing mice.
- Under the treatment regimens evaluated, GMI-1359 inhibited both tumor growth ($p<0.01$ vs. vehicle) and pulmonary metastases ($p<0.05$ vs. vehicle and DOX), with a trend towards reduction in tumor induced bone lesions.

Conclusion

- Our data provide a biologic rationale for the use of a dual E-selectin/CXCR4 inhibitor in children and young adults with osteosarcoma.
- Given its complementary mechanism of action to traditional chemotherapy, GMI-1359 warrants further development not only in osteosarcoma, but also in other malignancies where (1) primary tumor originates in the bone or (2) the tumor is likely to spread within the bone and/or to other organs (e.g. lung).