

Abstract

In the current studies we investigated if a dual E-selectin/CXCR4 antagonist (GMI-1359) could impact the intraosseous growth of the metastatic, androgen-independent PC3M cell line and affect chemosensitivity to docetaxel. PCa cells, including PC3M, selected for increased visceral and bone metastatic potential express high levels of E-selectin ligands and CXCR4 as compared to nonmetastatic PCa cell lines. We evaluated the ability of GMI-1359 administered alone or in combination with docetaxel to inhibit the growth and metastasis of intratibially implanted luciferase-transfected PC3M cells. Approximately two weeks post tumor cell implantation, mice were treated by intraperitoneal injection for 2 weeks with either saline twice daily; 40 mg/kg GMI-1359 twice daily, 5 mg/kg docetaxel once weekly or a combination of GMI-1359 and docetaxel. Thirty-five days after initiation of treatment, the percentage of tibiae positive by X-ray and the size of osteolytic lesions was impacted by treatment with GMI-1359 alone or in combination with docetaxel. Docetaxel alone had only a modest impact on intraosseous lesions. Lytic units were reduced by 38%, 78% and 88% in mice treated with docetaxel alone, GMI-1359 alone, or GMI-1359 in combination with docetaxel, respectively. The significantly reduced intraosseous growth of PC3M cells correlated with decreased serum levels of both mTRAP and type I collagen fragments.

Background

- Prostate cancer preferentially metastasizes to the skeleton where the bone microenvironment can stimulate tumor cell growth and spread, and promote the emergence of clinically-resistant disease.
- An improved understanding of the complex relationship between prostate carcinoma (PCa) cells and the bone microenvironment has created a powerful opportunity to develop novel therapies.
- PCa cells preferentially roll and adhere on bone marrow vascular endothelial cells, where constitutive E-selectin expression and abundant stromal cell-derived factor-1 α (SDF-1 α) are expressed and interact with E-selectin ligands and CXCR4, respectively, present on PCa cells.
- These molecular interactions may be targeted for pharmacologic treatments of bone metastatic disease.

Results

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

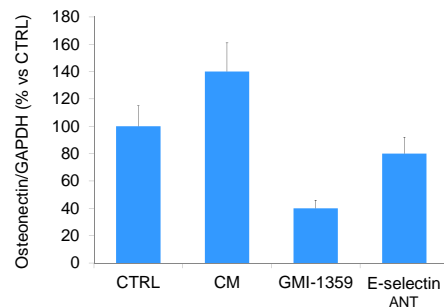
GMI-1359 (dual E-selectin/CXCR4 antagonist) and an E-selectin specific antagonist (ANT) were assessed for inhibition of (1) sialyl LeX binding to immobilized E-selectin and (2) CXCR4 binding to Raji cells. IC50's (μ M) were determined and summarized in Table 1.

Compound	E-selectin	CXCR4
GMI-1359	1.0	0.5
E-selectin ANT	2.4	>10000

Summary. The small molecule glycomimetic, GMI-1359, inhibits ligand binding to both E-selectin and CXCR4. Shown for comparison is an E-selectin specific ANT. GMI-1359 and E-selectin ANT were subsequently evaluated for anti-tumor activity in the E-selectin ligand and CXCR4 positive, PC3M prostate xenograft model.

Figure 1. Production of osteonectin in mouse stromal cell cultures following incubation in conditioned media of PC3M cells treated with GMI-1359

Stromal cell cultures were derived from calvaria of 6- to 9-day-old CD1 mice. The bone samples were sequentially digested with 1 mg/mL type IV collagenase and 0.025% trypsin. Cells were maintained in DMEM with 10% FBS. Stromal cell cultures were incubated for 24 h with conditioned media obtained from PC3M cells treated with GMI-1359 or E-selectin ANT for 24 h. Total RNA was extracted, reverse transcribed, and 0.1 μ g used for PCR reactions to determine Osteonectin.



Summary. Conditioned media from PC3M tumor cell cultures stimulates the production of osteonectin from stromal cells. Incubation of PC3M cells with GMI-1359 significantly attenuates production of osteonectin, under identical culture conditions.

Figure 2A. Treatment of intraosseous PC3M tumor by GMI-1359 either alone or in combination with docetaxel.

2 x 10⁵ Luc enabled PC3M cells were injected into the proximal tibiae of 4-week old male CD1 nu/nu mice. Tumor progression was monitored by bioluminescence analyses. Fourteen days post tumor cell injection, mice were randomized (n=8/group) and treated with: saline alone, docetaxel alone (5 mg/kg iv qwx3), GMI-1359 or E-selectin ANT alone (40 mg/kg ip bidx21 days), or the combination of docetaxel and GMI-1359 or E-selectin ANT. On study day 35, the development and extent of osteolytic lesions was evaluated by digital examination of radiographs (ImageJ) and lytic units/tibia was calculated.

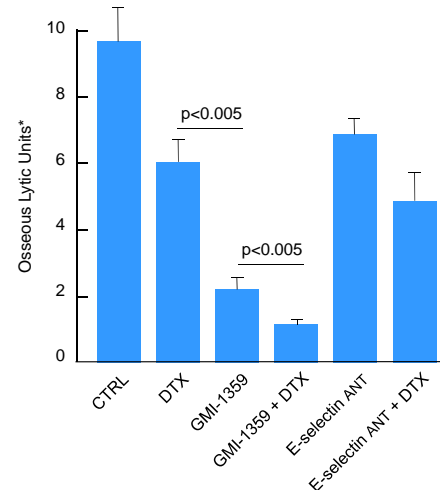
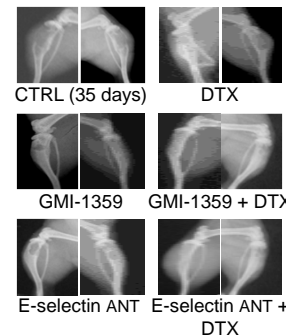


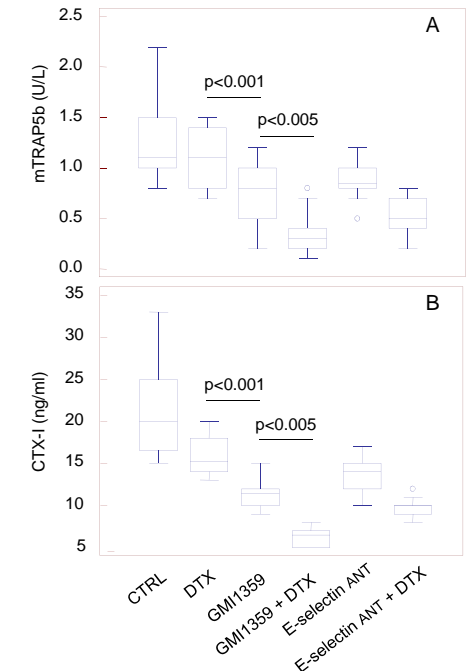
Figure 2B. Hindlimb radiographs from PC3M-bearing mice treated with GMI-1359 either alone or in combination with docetaxel.



Summary. Compared to DTX, treatment with GMI-1359, alone or in combination with DTX, lead to a significant reduction in growth of intraosseous PC3M tumor.

Figure 3. Serum mTRAP (A) and CTX-1 (B) levels in tumor bearing mice treated with GMI-1359 alone or in combination with docetaxel.

Blood samples were collected from treated mice on study day 35. Measurement of mouse cross linked C-telopeptide of type I collagen (CTX-1) and mouse TRAP-5b (mTRAP5b) were determined by ELISA.



Summary. Compared to DTX treatment, serum levels of CTX-1 and mTRAP5b, two specific markers of bone reabsorption, were significantly lower in mice treated with GMI-1359 alone or in combination with DTX.

Conclusion

- Our data provides a strong biologic rationale for the use of a dual E-selectin/CXCR4 inhibitor as an adjuvant to taxane-based chemotherapy in men with high-risk prostate cancer to prevent growth of bone metastases.
- Given its complementary mechanism of action to traditional chemotherapy, GMI-1359 warrants further development not only in prostate carcinoma, but also in other malignancies where tumor cells are likely to spread to bone.