Abstract # 428

DUAL E-SELECTIN AND CXCR4 INHIBITION REDUCES TUMOR GROWTH AND INCREASES THE SENSITIVITY TO DOCETAXEL IN EXPERIMENTAL BONE METASTASES OF PROSTATE CANCER

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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-1α (SDF-1α) 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.

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Results

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

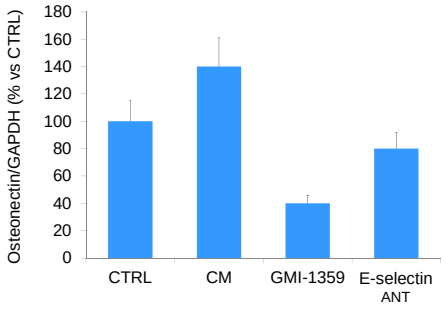
GMI-1359 (dual E-selectin/CXCR4 antagonist) and an E-selectin specific antagonist (ANT) were assessed for inhibition of (1) sialyl LeX binding to immobilized E-selectin and (2) CXCR4 binding to Raji cells. IC50's (μM) were determined and summarized in Table 1.

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, reversed transcribed, and 0.1 μg 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 10^5 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.

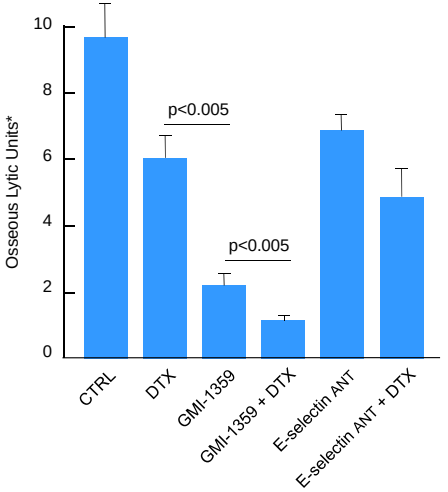
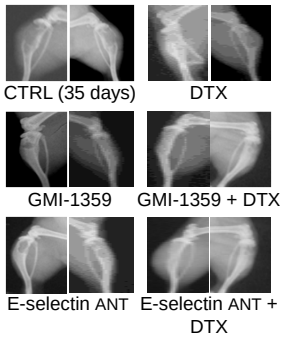


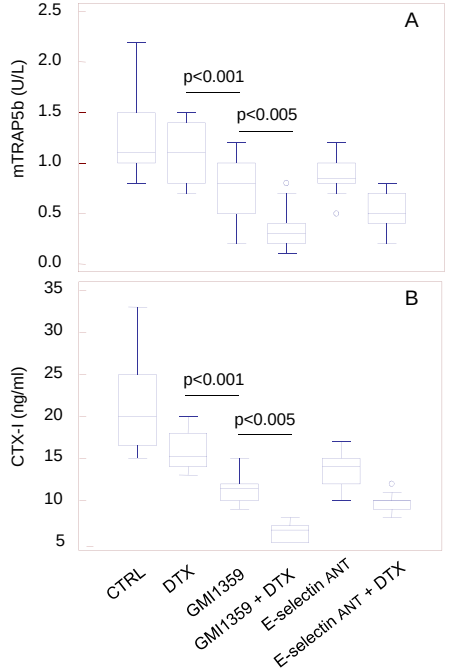
Figure 2B. Hindlimb radiographs from PC3M-bearing mice treated with GMI-1359 either alone or in combination with docetaxel.



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-telpeptide 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.