A small molecule glycomimetic antagonist of E-selectin and CXCR4 (GMI-1359) delays pancreatic tumor metastasis and significantly alters the pancreatic tumor microenvironment

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William E. Fogler, PhD, John L. Magnani, PhD, and Michael A. Hollingsworth, PhD
I have the following financial relationships to disclose:
Grant/Research support from: GlycoMimetics, Inc.

I will discuss the following off label use and/or investigational use in my presentation: GMI-1359 (E-selectin and CXCR4 dual antagonist) developed by GlycoMimetics, Inc.
Inhibitors

• GMI-1359: small molecule antagonist of E-selectin and CXCR4 developed by GlycoMimetics

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>E-selectin IC50</th>
<th>CXCR4 IC50</th>
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<tbody>
<tr>
<td>GMI-1359</td>
<td>1.0 uM</td>
<td>0.5 uM</td>
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E-selectin, an endothelial adhesion protein

- Expressed on vascular and lymphatic endothelial cells under inflammatory conditions ("activated" endothelium)
- Binds to sialyl Lewis a and X carbohydrate structures found on leukocytes and tumor cells

Function: to mediate the adhesion of leukocytes to endothelial cells for migration across blood and lymphatic vasculature

Tumor cells co-opt this function
CXCL12-CXCR4 Chemokine Axis
CXCL12-CXCR4 Chemokine Axis Metastatic Pancreatic Adenocarcinoma (PDAC)

- CXCR4 overexpression correlates with increased PDAC progression and metastasis (Marchesi et al., 2004. Cancer Res, 64.)
- CXCR4 blockade inhibits PDAC cell migration and invasion (Mori et al., 2004. Mol Cancer Ther., 3.)
- Dual inhibition of CXCR4 and PD-L1 improved T cell infiltration and reduced tumor growth in a spontaneous mouse model of PDAC (Feig et al., 2013. PNAS, 110(50)).
- This suggests that both CXCR4 and E-selectin contribute to PDAC metastasis and are potential targets for therapy – GMI-1359
Blockade of E-selectin and CXCR4 inhibits PDAC invasion of a lymphatic endothelium

1. Plate a confluent monolayer of LECs in the insert of a Boyden chamber.
2. Add fluorescently labeled PDAC cells to upper insert and a chemoattractant to the lower wells.
3. Allow PDAC cells to transendothelial migrate for 24 hrs.
4. Remove non-migrated tumor cells from inside of the insert and quantify the migrated cells.
Blockade of E-selectin and CXCR4 inhibits PDAC invasion of a lymphatic endothelium

**E-selectin Antagonist**

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<td>50 ug/ml</td>
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**GMI-1359**

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**sLeα**

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**FL1H-AF488**

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**In Vivo Inhibition of E-selectin and CXCR4 - Metastasis**

**Treatment Groups (n=15)**

- **Group 1:** Vehicle Control (daily)
- **Group 2:** E-selectin antagonist (40 mg/kg daily)
- **Group 3:** GMI-1359 (40 mg/kg daily)
- **Group 4:** Gemcitabine (100 mg/kg every 4 days)
- **Group 5:** E-selectin antagonist and Gemcitabine
- **Group 6:** GMI-1359 and Gemcitabine
Effects of E-selectin/CXCR4 Inhibition on Metastasis
GMI-1359 Modifies the Pancreatic Tumor Microenvironment - Desmoplasia

αSMA

Vehicle
E-selectin Antagonist
GMI-1359

* p < 0.05
** p < 0.01
GMI-1359 Modifies the Pancreatic Tumor Microenvironment – Lymphatic Vascular Density

**LYVE-1**

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<tr>
<td>E-selectin Antagonist</td>
<td>7 ± 2</td>
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<tr>
<td>GMI-1359</td>
<td>10 ± 1</td>
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* p < 0.05  
** p < 0.01
GMI-1359 Modifies the Pancreatic Tumor Microenvironment – CD45+ Infiltrates

Vehicle

E-selectin Antagonist

GMI-1359

Tumor-Infiltrating CD45+ Cells

CD45+ Aggregates
These data regarding changes to the pancreatic tumor microenvironment lead us to evaluate the relationship between desmoplasia and lymphatic vessels with a focus on the role of the CXCL12/CXCR4 axis.
Pancreatic fibroblasts promote lymphatic endothelial migration through CXCL12/CXCR4

**CXCL12 (pg/ml)**

```
Pancreatic fibroblast CM
```

```
Panc. Fibroblast CM
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* p < 0.05
** p < 0.01
*** p < 0.001
**** p < 0.0001
We also wanted to evaluate whether pancreatic fibroblasts, through CXCL12, may influence the ability of lymphatic endothelial cells to facilitate tumor invasion of the endothelium.
CXCR4 ligand, CXCL12, promotes the ability of lymphatic endothelial cells to facilitate PDAC transendothelial invasion.

* p < 0.05
*** p < 0.0005
Conclusions:

• Blocking E-selectin and CXCR4 through GMI-1359 inhibits PDAC transendothelial migration delays pancreatic tumor metastasis

• GMI-1359 significantly alters the composition of pancreatic tumor microenvironment – desmoplasia and lymphatic vascular density

• Pancreatic fibroblasts attract lymphatic endothelial cells via CXCL12/CXCR4 as well as increase the ability of lymphatic endothelial cells to support PDAC transendothelial invasion
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