Inhibition of E-Selectin or E-selectin together with CXCR4
Re-sensitizes Multiple Myeloma to Treatment

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- and -

I will not discuss off label use and/or investigational use in my presentation.
Multiple myeloma (MM)

- Plasma cell malignancy localized mainly in the bone marrow
- Characterized by metastasis of MM cells across the skeletal system
- The progression of MM involves a continuous **egress** (re-circulation) of the tumor cells in the peripheral blood and **homing** (re-entrance) into the bone marrow

Cellular trafficking in hematologic malignancies

Areas of active myeloma (PET scan)

Lu et al 2012
The supportive role of the bone marrow microenvironment in MM

- The interactions of tumor cells with their bone marrow microenvironment facilitate **tumor progression**, **metastasis** and **drug resistance**

**Cellular compartment:**
- Accessory Cells
- Blood vessels

**Non-cellular compartment:**
- Extracellular Matrix
- Soluble factors

Azab AK et al. *Blood* 2012;119:1468-1478 (Selectins and endothelial cells)
Azab AK et al. *Blood* 2009;113:4341-4351 (Chemokines an stroma)
Azab AK et al. *Blood* 2009;114:619-29 (ECM and stroma)
De la Puente P et al. *Biomaterials* 2015 (3DTEBM)

Muz B et al. *Blood Cancer J* 2015 (Hypoxia)
McMillin DW et al. *Nat Med* 2010;16:483-9 (Stroma)
Fulciniti M et al. *Clin Cancer Res* 2009;15:7144-52 (IL-6)
Targeting cell trafficking as a strategy to sensitize MM cells

**Blood Vessel**

- Rolling
- Adhesion
- Extravasation

**Endothelium**

- Signaling Pathways

**Bone Marrow**

- Homing

**Stroma**

- Stromal cells

**Chemokines**

- CXCR4 : SDF-1

**Selectins**

- E-selectin : CLA

**Integrins**

- VLA4

**RTK’s**

- Eph-B2
- FGFR3

**Extracellular Matrix**

- Fibronectin
- VCAM

- Targeting cell trafficking as a strategy to sensitize MM cells

**Signaling Pathways**

- Eph-B2
- FGFR3
- Selectins
- Integrins
- RTK's
- Extracellular Matrix
Aim

To test the role of E-selectin (GMI-1271) and E-selectin/CXCR4 (GMI-1359) antagonists on MM cell trafficking *in vitro* and *in vivo* as a potential approach to overcome bone marrow microenvironment-induced drug resistance
Expression of cell surface molecules in endothelial, stromal and myeloma cells

HUVECs – human umbilical vascular endothelial cells
SDF-1 – stromal-derived growth factor-1
HS5 – stromal cell line (normal)
In the presence of SDF-1, GMI-1359 inhibits MM cell chemotaxis more effectively than GMI-1271.

**METHOD**

**Conditioned media:**
- HUVECs
- HS5
- MSP1

**MM.1S cells pre-treated with GMI-1271 or GMI-1359 for 1hr**

**Boyden Chamber**

**Graph:**
- # of MM.1S cells migrated (Relative to untreated)

- Bars for HUVECs media, SDF1 50nM, HS5 media, MSP1 media

**Abbreviations:**
- HUVECs – human umbilical vascular endothelial cells
- SDF1 – stromal-derived growth factor-1
- HS5 – stromal cell line (normal)
GMI-1359 inhibits MM cell trans-endothelial migration more effectively than GMI-1271, especially under hypoxic conditions.

METHOD

Labeled MM cells
Cultured in Normoxia or Hypoxia (1%O2) for 24hrs
Pre-treated with GMI-1271 or GMI-1359 for 1hr

Trans-Endothelial Migration (% of untreated normoxic MM cells)

- Red bars: Normoxia
- Blue bars: Hypoxia

HUVECs
MSP1
Normoxic RPMI8226
Hypoxic RPMI8226

Barbara Muz
4 April 2017
GMI-1359 inhibits extravasation of MM cells to the bone marrow in vivo

METHOD

MM Untreated (Calcein Violet)

MM GMI-1271 (Calcein Orange)

MM GMI-1359 (Calcein Green)

Mix

IV injection

Sacrifice at 90 mins Collect Blood

Analyze by flow cytometry

Number of circulating MM cells (in 10,000 MNCs)

0 50 100 150 200 250

Untreated GMI-1271 (20μM) GMI-1359 (20μM)
GMI-1271 in combination with lenalidomide overcomes stroma-induced drug resistance *in vitro* and inhibits tumor growth *in vivo*

**In vitro** – MTT assay

**In vivo** - Human Xenograft Disseminated Mouse Model
GMI-1271 in combination with carfilzomib (CFZ) overcomes stroma-induced drug resistance *in vitro* and prolongs mice survival

*In vitro – MTT assay*

*In vivo - Syngeneic 5TGM1 Disseminated Mouse Model*
GMI-1359 in combination with carfilzomib (CFZ) overcomes stroma-induced drug resistance *in vitro* and prolongs mice survival

*In vitro – MTT assay*

*In vivo - Syngeneic 5TGM Disseminated Mouse Model*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>MST (days)</th>
<th>P vs saline</th>
<th>P vs CFZ</th>
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<tr>
<td>saline</td>
<td>10</td>
<td>32.5</td>
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<td>GMI-1359 40 mg/kg IP QDx14</td>
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<td>GMI-1359 + CFZ</td>
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<td>49</td>
<td>0.001</td>
<td>0.014</td>
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</table>
Summary

- Endothelial cells (HUVECs) and stromal cells (MSP-1 and HS5) express high levels of E-selectin
- CXCR4 is highly expressed on MM cell lines; CLA is highly expressed in RPMI8226

- *In vitro*, MM cell adhesion, chemotaxis, and trans-endothelial migration is decreased by GMI-1271 and even further by GMI-1359, in the presence of SDF-1

- *In vivo*, GMI-1359 significantly inhibits extravasation of MM cells to the bone marrow

- *In vitro*, GMI-1271 and GMI-1359 combined with either lenalidomide or carfilzomib overcome stroma-mediated drug resistance

- *In vivo*, GMI-1271 in combination with lenalidomide reduces tumor growth
- Mice survival is prolonged by GMI-1271 combined with carfilzomib, and even further by GMI-1359 combined with carfilzomib
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Azab Lab

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Thank you for your attention!