Treatment of Acute Vaso-occlusion in Mouse Models of Sickle Cell Disease following Intravenous or Subcutaneous Administration of a Highly Potent E-selectin Specific Inhibitor

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John L. Magnani and William E. Fogler are employees and stockholders of GlycoMimetics, Inc.

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Well-Characterized Mechanisms Critical to Inflammatory Response Driving VOC

- First step in extravasation from bloodstream—selectins bind immune cells to endothelium
- Neutrophils and monocytes are activated when they bind to selectins
  - Changes β2 integrins to high affinity conformation
  - Allows subsequent adhesion in the extravasation process and adhesion to other cells
- Activation through selectins also leads to production of microparticles from neutrophils, rich in tissue factor, and can promote thrombus formation

Development of VOC

- Blood vessel
- Endothelial cells
- Red blood cell
- Sickled red blood cells
- Blood flow
- Rolling white blood cell
- Selectin
- Adherent white blood cell
- Vascular occlusion
E-selectin Plays a Dominant Role in Sickle Cell VOC

Inhibiting E-selectin Catch Bonds

Blocking only E-selectin, not P-selectin, Fully Inhibits RBC binding to Immobilized Leukocytes During VOC

Rolling on E-selectin, not P-selectin, Activates Arrest and Immobilization

Soluble E-selectin, Not Soluble P-selectin, Correlates with Poor Survival (P = 0.002)

In contrast, "...mortality was negatively and not significantly related to log10 sE-selectin values (P = 0.38)"


E-selectin Binding to the Carbohydrates (Sialyl Le^x) Expressed on L-selectin Induces High Affinity Conformation of b2-Integrin both Directly and Through TLR4

**E-selectin Plays a Dominant Role in VOC**

Selectin catch-bonds mechanotransduce integrin activation and neutrophil arrest on inflamed endothelium under shear flow

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E-selectin inhibition blocks the conformational change to High Affinity Integrin receptors both directly and through MRP8/14 signaling
Binding Constant of GMI-1687 for E-selectin as Determined by Surface Plasmon Resonance

\[ K_D = 2.3 \text{ nM} \]

<table>
<thead>
<tr>
<th>GMI 1687 conc (nM)</th>
<th>Req (RU)</th>
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<tbody>
<tr>
<td>0.000</td>
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<tr>
<td>0.500</td>
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<td>40.000</td>
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<table>
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<tr>
<th>One site – Specific binding</th>
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<td>Best-fit values</td>
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<td>Std. Error</td>
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<td>95% Confidence Intervals</td>
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<td>Bmax</td>
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<tr>
<td>Kd</td>
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R square 0.9944
GMI-1687 is Totally Bioavailable through a Subcutaneous Dose

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<th>Route</th>
<th>Dose (mpk)</th>
<th>$T_{1/2}$ (hr)</th>
<th>MRT (hr)</th>
<th>$C_{\text{max}}$ (ng/mL)</th>
<th>$T_{\text{max}}$ (hr)</th>
<th>CI (L/hr/kg)</th>
<th>$V_{ss}$ (L/kg)</th>
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<td>SC</td>
<td>5</td>
<td>2.9</td>
<td>1.5</td>
<td>5127</td>
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<td>-</td>
<td>-</td>
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</tbody>
</table>
Assessment of GMI-1687 for Attenuation of Vaso-Occlusion in Nude Mice Given Human SSRBCs

TNFα 0.5 mg i.p. → Saline or GMI-1687

0 30 60 120 150 180 240

Fluorescence-labeled (rhodamine 6G) Human SSRBCs

Recording of window chamber (cremaster muscle)

Parameters
- SSRBC adhesion
- Blood flow
- Vessel occlusion

R. Zennadi, Duke University
Comparative Activity of GMI-1687 following IV or SC Administration on Adherent hSSRBCs in Inflamed Venules

R. Zennadi, Duke University
Comparative Activity of GMI-1687 following IV or SC Administration on Blood Flow in Inflamed Venules

R. Zennadi, Duke University

GMI-1687 IV

GMI-1687 SC

- Normal blood flow
- Slow blood flow
- Occluded vessel
Assessment of GMI-1687 for Attenuation of Vaso-Occlusion in the Townes Mouse Model of Human SCD

- The Townes mice have a transgene containing normal human α, γ, δ globins and sickle β globin and targeted deletions of murine α & β globins (α-/-, β-/-). This mouse model of SCD expresses exclusively human sickle hemoglobin.
  - ~100% sickle RBCs
  - Erythrocytes have significantly decreased osmotic fragility and increased dynamic rigidity
  - Anemic with hematocrits ~65% of WT mice
  - Baseline inflammation as evidence by vascular congestion, atrophy, fibrosis, and infarct found in lungs, liver, spleen and kidneys

R. Zennadi, Duke University
GMI-1687 Attenuates Sickle RBC Adhesion (A) and Vessel Occlusion (B) in the Townes Mouse Model of Human SCD

R. Zennadi, Duke University
Summary

- E-selectin plays a dominant role initiating the vaso-occlusive crisis in sickle cell disease
- GMI-1687 is a highly potent small molecule antagonist for E-selectin with a binding constant ($K_D$) of 2.3 nM
- GMI-1687 is completely bioavailable through subcutaneous dosing opening up the possibility of self administration.
- In a mouse model of vaso-occlusive crisis using human sickle rbc’s in nude mice, GMI-1687 blocks adherence of these cells and normalizes blood flow.
- In a transgenic mouse model containing human sickle hemoglobin (Townes mice), treatment with GMI-1687 inhibits induced vaso-occlusive crisis by blocking adherence of cells and normalizing blood flow.
- GMI-1687 contains desired properties that are compatible with very early treatment of a crisis outside the hospital setting.
Thank you

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