The E-selectin Inhibitor GMI-1687 Restores Normal Blood Flow in a Mouse Model of Sickle Cell Vaso-occlusion

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CSO & SVP
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**
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

- 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 sP-selectin values ($P = 0.36$)”*


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

- E-selectin but not P-selectin induces transition from rolling to arrest.
- Blocking L-selectin but not PSGL-1 abrogates arrest.


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


E-selectin Binding to the Carbohydrates (Sialyl Le\textsuperscript{x}) Expressed on L-selectin Induces High Affinity Conformation of β2-Integrin both Directly and Through TLR4

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

![Diagram showing the role of E-selectin in VOC](image)

E-selectin inhibition blocks the conformational change to High Affinity Integrin receptors both directly and through MRP8/14 signaling

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

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GlycoMimetics, Inc.
Binding Constant of GMI-1687 for E-selectin as Determined by Surface Plasmon Resonance

**K_D** = 2.3 nM

<table>
<thead>
<tr>
<th>GMI 1687 conc (nM)</th>
<th>Req (RU)</th>
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<tbody>
<tr>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>0.500</td>
<td>7.800</td>
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<td>16.000</td>
<td>54.000</td>
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<tr>
<td>40.000</td>
<td>57.100</td>
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</tbody>
</table>

Best-fit values:
- **Bmax**: 62.07
- **Kd**: 2.278

Std. Error:
- **Bmax**: 1.321
- **Kd**: 0.2208

95% Confidence Intervals:
- **Bmax**: 58.84 to 65.30
- **Kd**: 1.738 to 2.819

**R square**: 0.9944
GMI-1687 is Totally Bioavailable through a Subcutaneous Dose

GMI-1687
E-Sel $K_D = 2.3$ nM

<table>
<thead>
<tr>
<th>Route</th>
<th>Dose (mpk)</th>
<th>$T_{1/2}$ (hr)</th>
<th>MRT (hr)</th>
<th>$C_{max}$ (ng/mL)</th>
<th>$T_{max}$ (hr)</th>
<th>Cl (L/hr/kg)</th>
<th>$V_d$ (L/kg)</th>
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<tbody>
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<td>22209</td>
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<td>0.62</td>
<td>0.28</td>
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<tr>
<td>SC</td>
<td>5</td>
<td>2.9</td>
<td>1.5</td>
<td>5127</td>
<td>0.67</td>
<td>-</td>
<td>-</td>
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</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
- 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

GMI-1687 IV

- **Vehicle**: Adherent hSSRBCs (RFU) = 1.00
- **40 μg/Kg**: Adherent hSSRBCs (RFU) = 0.75
- **80 μg/Kg**: Adherent hSSRBCs (RFU) = 0.50

***P<0.001

GMI-1687 SC

- **Vehicle**: Adherent hSSRBCs (RFU) = 1.00
- **40 μg/Kg**: Adherent hSSRBCs (RFU) = 0.75
- **80 μg/Kg**: Adherent hSSRBCs (RFU) = 0.50

****P<0.0001

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

GMI-1687 IV

GMI-1687 SC

- Normal blood flow
- Slow blood flow
- Occluded vessel

R. Zennadi, Duke University
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

**PE-anti-mouse TER119**

![Graph showing experiment timeline](image)

**TNFa (0.5 μg i.p.)**

**Saline or GMI-1687 IV**

Recording of window chamber
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.