Inclacumab, a Fully Human Anti-P-Selectin Antibody, Directly Binds to PSGL-1 Binding Region and Demonstrates Robust and Durable Inhibition of Cell Adhesion

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Disclosures

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Inclacumab is a novel, fully human IgG4 monoclonal antibody that selectively targets P-selectin. It has safely demonstrated sustained (>12 weeks) anti-cell-adhesion effects in >700 individuals, including both healthy volunteers and patients with cardiovascular disease.\(^3\)-\(^6\)

Crizanlizumab, an FDA-approved humanized IgG2 monoclonal antibody, reduces the frequency of VOCs in patients with SCD by binding to P-selectin and blocking its interaction with the ligand PSGL-1.\(^2\)

Patients with SCD commonly present with VOCs caused by the adhesion of leukocytes and sickled red blood cells to the endothelium of blood vessels, with clinical manifestations of extreme pain, vascular obstruction, and tissue ischemia.\(^1\)-\(^2\)

**Objective**

To elucidate differences between inclacumab and crizanlizumab via in vitro functional assays

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Inclacumab Directly Binds to an Epitope in the PSGL-1 Binding Region of P-Selectin

The P-selectin inclacumab Fab interface

Overlap of binding sites of inclacumab and PSGL-1 on P-selectin

Fab, fragment antigen binding; PSGL-1, P-selectin glycoprotein ligand-1.
Inclacumab Has a Distinct Epitope from Crizanlizumab That Directly Overlaps the PSGL-1 Binding Site on P-Selectin

A. The interface of P-selectin and the ligand PSGL-1. B. The interface of P-selectin and the inclacumab antibody. C. The interface of P-selectin and the crizanlizumab antibody.

PSGL-1, P-selectin glycoprotein ligand-1.
Inclacumab Demonstrates Comparable Binding Affinity of P-Selectin but Longer Residence Time Compared with Crizanlizumab

A. Kinetics and affinity were analyzed using a Biacore T200. Recombinant P-selectin protein was passed over an immobilized inclacumab or crizanlizumab surface, generating data for both dissociation ($k_d$) and association ($k_a$). The value for $K_D$ was calculated as $k_d / k_a$. Relative binding was determined by parallel line analysis. Average kinetic values ($\pm$ SEM) measured from three independent experiments are listed.

<table>
<thead>
<tr>
<th></th>
<th>$k_a$ (M$^{-1}$s$^{-1}$)</th>
<th>$k_d$ (s$^{-1}$)</th>
<th>$K_D$ (M)</th>
<th>Relative binding</th>
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<tbody>
<tr>
<td>Inclacumab</td>
<td>4.07 (±0.09) 10$^5$</td>
<td>3.97 (±0.03) 10$^{-3}$</td>
<td>9.91 (±0.30) 10$^{-9}$</td>
<td>100%</td>
</tr>
<tr>
<td>Crizanlizumab</td>
<td>12.33 (±0.88) 10$^5$</td>
<td>11.0 (±0.01) 10$^{-3}$</td>
<td>9.05 (±0.33) 10$^{-9}$</td>
<td>68.9% (±2.1%)</td>
</tr>
</tbody>
</table>

B. Representative fitted binding sensorgrams for inclacumab or crizanlizumab P-selectin interactions where binding data were collected for each antibody at multiple concentrations.

C. Fitted dose-response curves for relative binding analysis showed a noticeable shift in the binding EC$_{50}$ and relative binding.

A. Kinetics and affinity were analyzed using a Biacore T200. Recombinant P-selectin protein was passed over an immobilized inclacumab or crizanlizumab surface, generating data for both dissociation ($k_d$) and association ($k_a$). The value for $K_D$ was calculated as $k_d / k_a$. Relative binding was determined by parallel line analysis. Average kinetic values ($\pm$ SEM) measured from three independent experiments are listed. B. Representative fitted binding sensorgrams for inclacumab or crizanlizumab P-selectin interactions where binding data were collected for each antibody at multiple concentrations. C. Fitted dose-response curves for relative binding analysis showed a noticeable shift in the binding EC$_{50}$ and relative binding.
Inclacumab Potently Suppresses the Interaction of P-Selectin with Its Ligand PSGL-1

A. Biacore competitive ligand-binding assay

B. Inhibition curve of pooled human sera

A. The competitive ligand binding assay to quantify P-selectin inhibitory activity in the Biacore platform, wherein human serum samples treated with different concentration ranges of antibodies were injected over the immobilized GSnP-6 (PSGL-1 binding domain) on the sensor chip in the presence of excess P-selectin protein, and inhibitory activity of the mAbs was measured as change from the baseline samples.

B. Inclacumab and crizanlizumab inhibition curves in pooled normal human serum.

mAb, monoclonal antibody; PSGL-1, P-selectin glycoprotein ligand-1.
Inclacumab Inhibits P-Selectin–Mediated Adhesive Functions in Cell-Based Assays

A. Static adhesion assay

B. Flow adhesion assay using blood from patients with SCD

A. Concentration ranges of inclacumab and crizanlizumab were tested on the adhesion of leukocyte-like HL60 cells expressing PSGL-1 to immobilized P-selectin on the plate. Representative inhibition curves are given.

B. Inclacumab and crizanlizumab significantly inhibited the adhesion of whole blood cells or leukocytes of patients with SCD (N=4) to immobilized P-selectin in a human ex vivo flow system.1

IC₅₀, half maximal inhibitory concentration; PSGL-1, P-selectin glycoprotein ligand-1; SCD, sickle cell disease.

1. Lui K, et al. Poster presented at: American Society of Hematology Annual Meeting; December 1-4, 2018; San Diego CA, USA.
Inclacumab Demonstrated Greater Maximal Platelet-Leukocyte Aggregate Inhibition in Response to Platelet Agonists

A. Platelet-leukocyte aggregate

B. Healthy volunteers

Patients with SCD

A. PLA assay reflects that cell-cell adhesion activities are elevated in patients with SCD.1  B. Human whole blood from healthy volunteers (N=6) or SCD patients (N=4) was spiked with inclacumab or crizanlizumab antibody concentrations prior to platelet activation. Inhibition of PLAs is given in percentage of total TRAP-induced PLA levels. Values represent means ± SEM.

ADP, adenosine diphosphate; PLA, platelet-leukocyte aggregate; Plt, platelet; SCD, sickle cell disease; TRAP, thrombin receptor activating peptide.

## Conclusions

Inclacumab directly binds to an epitope in the PSGL-1 binding region on P-selectin, competitively inhibiting the interaction of P-selectin and its ligand.

Inclacumab binds to human P-selectin with high affinity and potently suppresses the interaction of P-selectin with its ligand PSGL-1 and P-selectin–mediated cell adhesion in vitro.

Inclacumab demonstrated greater maximal platelet-leukocyte aggregate inhibition than crizanlizumab in blood samples from both healthy volunteers and patients with SCD.

Previously:

- In patients with coronary artery disease, IV administration of inclacumab was well tolerated at doses ≤20 mg/kg every 4 weeks.
- A single 20 mg/kg IV dose of inclacumab maintained maximum PLA inhibition for at least 12 weeks in healthy volunteers.
- Inclacumab properties allow for a longer and more convenient dosing interval in patients with SCD as compared with crizanlizumab.

**Inclacumab has the potential to reduce VOCs in sickle cell disease.**

Clinical studies of inclacumab in patients with SCD are planned for early 2021.

IV, intravenous; PLA, platelet-leukocyte aggregate; PSGL-1, P-selectin glycoprotein ligand-1; SCD, sickle cell disease; VOC, vaso-occlusive crisis.