Siglec-8, a Novel Selective Target for Eosinophilic Gastrointestinal Diseases (EGIDs), Found on Eosinophils and Mast Cells in Human Tissue

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BACKGROUND

• Pathologic accumulation and over-activation of mast cells and eosinophils are implicated in multiple chronic inflammatory diseases in the GI tract including eosinophilic esophagitis, gastritis, gastroenteritis, and colitis - collectively termed eosinophilic gastrointestinal disorders (EGIDs)
• Patients with EGIDs have debilitating symptoms such as dysphagia, abdominal pain, nausea, vomiting, and diarrhea

Figure 1. Pathogenesis of EGIDs (Illustrative)

• Eosinophilic gastritis (EG) and gastroenteritis (GEG) are EGIDs that affect 45,000 - 50,000 patients in the US, though this number may be significantly underestimated
• Current treatment options such as diet restriction and corticosteroids have limited efficacy and/or are inappropriate for chronic use
• There is a significant medical unmet need for novel therapies

Figure 2. AK002 Mechanism of Action

METHODS

• Single-cell suspensions were prepared by enzymatic & mechanical digestion (Miltiary) of fresh biopsies from patients clinically diagnosed with EGIDs (n=9) or non-disease controls (n=12)
• Multi-color flow cytometry was performed to quantify immune cells and evaluate the activation state of eosinophils & mast cells
• Ex vivo activity of AK002 was evaluated against eosinophils and mast cells in dissociated EGID biopsies using Luminex (Millipore)

RESULTS

Figure 3. Study Design

Figure 4. Immune Cell Phenotyping Flow Cytometry Gating Strategy in EGID Biopsy Tissue

Figure 5. Increased Number of Eosinophils and Mast Cells in Esophagus of EoE Patients & Stomach of EG Patients

Figure 6. Siglec-8 is Selectively Expressed on Eosinophils and Mast Cells in EGID Human Tissue

Figure 7. Eosinophils and Mast Cells Are the Only Immune Cell Types Significantly Increased in EG Biopsies

Figure 8. Eosinophils and Mast Cells in EG Biopsies Display an Increased Activation State

CONCLUSIONS/DISCUSSION

• Eosinophils and mast cells are significantly increased in biopsy samples from patients diagnosed with EoE and EG
• Mast cells were elevated to the same extent as eosinophils in both EoE and EG biopsies
• Eosinophils and mast cells in EG biopsies display an activated phenotype, suggesting they may be pathogenic in EGIDs
• Siglec-8 is selectively expressed on eosinophils and mast cells at high levels in EGID biopsies
• AK002, a humanized Siglec-8 antibody, demonstrates anti-eosinophilic and mast cell inhibitory activity in EG biopsies
• Targeting Siglec-8 with AK002 may represent a novel approach to treat EGIDs

Eosinophils and mast cells in EG biopsies display an increased surface bound IgE, consistent with the atopic nature of EG

Table 1: AK002 activity

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Eosinophils</th>
<th>Mast Cells</th>
</tr>
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<tbody>
<tr>
<td>Non-Disease</td>
<td>10%</td>
<td>5%</td>
</tr>
<tr>
<td>Disease</td>
<td>20%</td>
<td>10%</td>
</tr>
</tbody>
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AK002 inhibits IgE-mediated mast cell cytokine production in ex vivo EG biopsies

Figure 9. AK002 Inhibits IgE-mediated Mast Cell Cytokine Production in ex vivo EG Biopsies

Ex vivo EG Tissue Supernatants

- EG biopsies contain an abundant IgE-primed mast cell population
- Crosslinking of the IgE receptor on mast cells results in VEGF and IL-4 production in dissociated EG biopsies
- VEGF and IL-4 play a role in recruiting inflammatory cells such as eosinophils
- These data suggest that IgE-activated mast cells could recruit eosinophils to the stomach mucosa in EG patients
- AK002 inhibits IgE-mediated VEGF and IL-4 production in dissociated EG biopsies

Figure 10: AK002 inhibits IgE-mediated mast cell cytokine production in ex vivo EG biopsies

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