EGID Biopsies Have Functionally Distinct and Activated Mast Cells That Contribute to Disease Pathogenesis and Are Inhibited By an Anti-Siglec-8 Antibody, Antolimab (AK002)

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Eosinophilic Gastrointestinal Diseases (EGIDs)

Eosinophilic Gastritis (EG)

Eosinophilic Esophagitis (EoE)

Eosinophilic Duodenitis (EoD)

ESOPHAGUS

STOMACH

DUODENUM

Chronic Eosinophilic Inflammation of the Esophagus, Stomach, or Duodenum

• Eosinophils and mast cells are important drivers of disease

• Symptoms: abdominal pain, nausea, early satiety, loss of appetite, bloating, abdominal cramping, vomiting, diarrhea, and dysphagia

• No FDA approved treatment for EoE, EG, or EoD

• Current standard of care: diet and/or steroids

Eosinophils and Mast Cells Drive EGID Pathogenesis

- EGIDs are characterized by antigen-mediated eosinophil and mast cell infiltration in the esophagus (EoE), stomach (EG), and duodenum (EoD).
- Despite the evidence of mast cell involvement in EGIDs, the mechanism by which they contribute to disease pathogenesis has yet to be established in human tissue.
Antolimab Targets Siglec-8 on Eosinophils & Mast Cells

Activating Receptors

Siglec-8

Cell Membrane

Mast cell

Eosinophil

Inflammatory response

 Activation

antolimab (AK002)

Inhibition

Mast cell

Eosinophil

ADCC/Apoptosis

Inhibition
Eosinophils and Mast Cells Are Increased in EoE, EG, and EoD Biopsies

Increased numbers of eosinophils and mast cells are found across EGIDs

** p<0.01; *** p<0.001
Mast Cells Are Activated in Upper and Lower EGIDs

Mast cell activation is seen in EoE, EG, and EoD biopsies.

![Graph showing mast cell activation](image)
Gating Strategy and Method for Sorting and Activating Mast Cells from GI Tissue

Sorted Mast Cells

- Unstimulated (Basal Production of Cytokines)
- PMA/Ionomycin (Induced Production of Cytokines)
EoE, EG, and EoD Mast Cells Basally Produce Th2 Inflammatory Mediators

Mast Cells

- **IL-5**: Normal EGID mast cells have significantly lower IL-5 levels compared to normal mast cells (**p<0.05**).
- **IL-13**: Normal EGID mast cells have significantly lower IL-13 levels compared to normal mast cells (**p<0.001**).
- **CCL3**: EGID mast cells produce significantly higher levels of CCL3 compared to normal mast cells (**p<0.001**).
- **TNFα**: No significant difference in TNFα levels between normal and EGID mast cells.

Mast cells from EGID tissue have a functionally distinct cytokine profile.
EGID Tissue Mast Cells Produce Increased Levels of Inflammatory Cytokines Upon Stimulation

Mast Cells

- **GM-CSF**
- **VEGF**
- **TNF-α**
- **IL-5**

Graphs show cytokine levels per cell for different mast cell types and conditions:

- GM-CSF: Non-Diseased GI Mast Cells vs. EGID Mast Cells
- VEGF: Non-Diseased GI Mast Cells vs. EGID Mast Cells
- TNF-α: Non-Diseased GI Mast Cells + PMA/I vs. EGID Mast Cells + PMA/I
- IL-5: Non-Diseased GI Mast Cells vs. EGID Mast Cells

- CCL3: Non-Diseased GI Mast Cells + PMA/I vs. EGID Mast Cells + PMA/I
- CCL4: Non-Diseased GI Mast Cells + PMA/I vs. EGID Mast Cells + PMA/I
- IL-13: Non-Diseased GI Mast Cells + PMA/I vs. EGID Mast Cells + PMA/I

Statistical significance:

- * p<0.05
- ** p<0.01
- *** p<0.001
Antolimab Inhibits Cytokine Production Induced by IgE/FcεRI Activation of EGID Biopsy Mast Cells

Isolate mast cells from EGID biopsies

Stimulate mast cells with α-FcεRI antibody

Evaluate mast cell inhibition by measuring cytokine production

0h 16h

VEGF

GM-CSF

IL-5

pg/mL

pg/mL

pg/mL

Unstimulated
ISO + αFcεRI
Antolimab + αFcεRI
Elevated and activated mast cells are found across EGIDs, including EoE, EG, and EoD.

- Mast cells from EGID tissue basally produce Th2 cytokines that are associated with T cell activation and eosinophil and mast cell recruitment.
- Upon stimulation, EGID mast cells produce higher levels of mediators compared to non-diseased GI tissue mast cells, suggesting mast cells from EGID tissue have increased pathogenic potential.
- IgE/FcεRI activation of EGID tissue mast cells induces mediator production that is inhibited by antolimab.
- Targeting both mast cells and eosinophils may be needed to significantly reduce inflammation in EGIDs.