EoE Biopsies have Elevated and Activated Mast Cells that Produce Cytokines and Chemokines that Drive Disease Pathogenesis

Melina Butuci1, Emily C. Brock1, Julia Schanin1, Alan Xu1, Henrik S. Rasmussen1, Bhupinder Singh1, Richard Drake2, Amy Holman2, Kathryn Peterson2, and Bradford A. Youngblood1

1Alatex Inc. Redwood City, CA; 2University of Utah, UT

BACKGROUND

- Eosinophilic gastrointestinal diseases (EGIDs) are a rare set of conditions characterized by the pathologic accumulation of eosinophils in the gastrointestinal tract.
- While eosinophils have been strongly associated with EGIDs, localized mast cells are also elevated in eosinophilic esophagitis, gastritis, and duodenitis.
- Despite evidence of mast cells being an important component of EGIDs, the mechanism by which they contribute to disease pathogenesis has yet to be established in human tissue.
-

METHODS

- Single-cell suspensions were prepared by enzymatic & mechanical digestion (Figure 3) of fresh biopsies from patients clinically diagnosed with EoE or non-disease control esophageal tissue.
- Multi-color flow cytometry was performed to quantify immune cells and evaluate the activation state of eosinophils & mast cells as shown in Figure 4.
- Mast cells were FACs-sorted from EoE biopsies or non-diseased GI tissues as shown in Figure 7 followed by overnight incubation with or without Lirentelimab.
- Cell-surface proteins were collected the following day and cytokines were quantified using meso scale discovery (MSD) system.
- The following cytokines were analyzed: IL-4, IL-5, IL-6, IL-9, IL-10, IL-13, IL-18, IL-33, GM-CSF, INFγ, TNFα, CCL2, CCL3, CCL4, CCL11, CCL17, and VEGF.

RESULTS

- Elevated and activated mast cells are found in patients with EoE.
- Mast cells from EoE tissue basally produced 2 cytokines that are associated with T cell activation and eosinophilic inflammation.
- Upon stimulation, EoE mast cells further produced abundant cytokines and chemokines that correlated with the percentage of tissue eosinophils, suggesting mast cells can directly recruit eosinophils to inflamed tissue.
- Therefore, targeting both eosinophils and mast cells may be needed to significantly reduce inflammation.

Figure 1. Pathogenesis of EGIDs

Figure 2. Lirentelimab (AK002) Mechanism of Action

Figure 3. Study Design

Figure 4. Flow Cytometry Gating Strategy for Mast Cells and Eosinophils in EoE Biopsy Tissue

Figure 5. Increased Numbers of Eosinophils and Mast Cells in EoE Biopsies

Figure 6. Resting Mast Cells Display an Increased Activation State in EoE Biopsies

Figure 7. Gating Strategy and Method for Activating Sorted Mast Cells from GI Tissue

Figure 8. Mast Cells from EoE Tissue Basally Produce IL-5, IL-13, and CCL3

Figure 9. EoE Tissue Mast Cells Produce Increased Levels of Cytokines upon Stimulation with PMA/Non agonist

Figure 10. Mast Cell-Derived GM-CSF and VEGF Correlate with Tissue Eosinophil Percentage in EoE Biopsies

Figure 11. Correlation scatter (Spearman) of GM-CSF and VEGF cytokine levels/cell cytokines measured from isolated EoE mast cells and the percentage of tissue eosinophils in the EoE biopsies

Figure 12. Cytokine level per mast cell in supernatants from overnight culture of EoE mast cells sorted with functional activity (black). Mast cell cytokine levels were calculated by dividing the cytokine concentration (pg/mL) by total mast cells plated. Individuals donors are plotted. * p<0.05; ** p<0.01; *** p<0.001.