Symptomatic Patients Suspected of Eosinophilic Gastritis and/or Gastroenteritis Have Elevated Mucosal Mast Cell Counts Without Eosinophilia

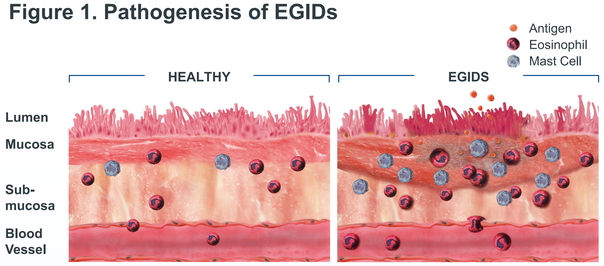
Joseph A. Murray[1], Evan S. Dellon[2], Kathryn A. Peterson[3], Gary W. Falk[4], Mirna Chehade[5], Robert M. Genta[6], Nirmala Gonsalves[7], Marc E. Rothenberg[8], Paneez Khoury[9], John Leung[10], Adam C. Bledsoe[1], Camilla Shaw[11], Henrik S. Rasmussen[11], Bhupinder Singh[11], Alan T. Chang[11], Bradford A. Youngblood[11], Amol P. Kamboj[11], Ikuo Hirano[7]

[1]Mayo Clinic Rochester, Rochester, MN; [2]University of North Carolina, Chapel Hill, NC; [3]University of Utah, Salt Lake City, UT; [4]University of Pennsylvania, Philadelphia, PA; [5]Icahn School of Medicine at Mount Sinai, NYC, NY; [6]Baylor College of Medicine, Houston, TX; [7]Northwestern University, Chicago, IL; [8]Cincinnati Children's Hospital Medical Center, Cincinnati, OH; [9]NIH/NIAID, Bethesda, MD; [10]Tufts University Medical Center, Boston MC; [11]Allakos, Inc., Redwood City, CA

BACKGROUND

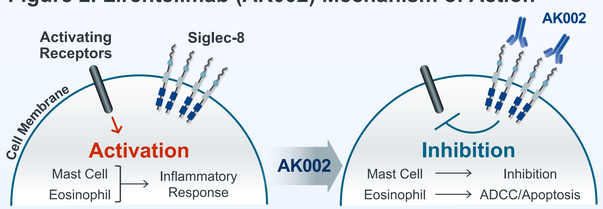
- Pathologic accumulation and over-activation of eosinophils are implicated in multiple chronic inflammatory diseases in the GI tract including eosinophilic esophagitis (EoE), gastritis (EG), gastroenteritis (EGE), and colitis - collectively termed eosinophilic gastrointestinal diseases (EGIDs)
- Patients with EGIDs have decreased quality of life due to debilitating symptoms such as dysphagia, abdominal pain, nausea, vomiting, and diarrhea
- While the pathogenesis of EGIDs has historically been thought to be driven by eosinophils, mast cells have also been shown to be elevated in EoE[1,2]
- The role of mast cells in other EGIDs has yet to be established

Figure 1. Pathogenesis of EGIDs



- EG and EGE affect 45,000 - 50,000 patients in the US, though this number may be significantly underestimated[3]
- Current treatment options such as diet restriction and corticosteroids have limited efficacy and/or are inappropriate for chronic use
- There is a significant unmet need for novel targeted therapies

Figure 2. Lirentelimab (AK002) Mechanism of Action



- Siglec-8 is an inhibitory receptor selectively expressed on human eosinophils and mast cells, and therefore represents a novel target for the treatment of EGIDs
- Lirentelimab (AK002) is a novel, humanized, non-fucosylated IgG1 monoclonal antibody to Siglec-8
- Engagement of Siglec-8 receptor by lirentelimab triggers:
  - Antibody dependent cell mediated cytotoxicity (ADCC) against eosinophils (blood)
  - Inhibition of mast cells and apoptosis of tissue eosinophils (tissue)
- ENIGMA, a Phase 2 multi-center, randomized, double-blind, placebo-controlled study of lirentelimab, represents the largest clinical trial of patients with EG and/or EGE

OBJECTIVE

- To characterize symptomatic patients with suspected EG/EGE who did not meet histopathologic entry criteria for mucosal eosinophilia for a Phase 2 randomized, double-blind, placebo-controlled study of lirentelimab in patients with EG/EGE

METHODS

Figure 3. ENIGMA Screening Protocol

Symptom PRO

- Subjects with prior diagnosis or suspected EG/EGE entered screening
- Subjects with an average weekly score of ≥3 intensity (0-10 scale) for abdominal pain, diarrhea and/or nausea for ≥2 weeks on a PRO questionnaire qualified for an upper endoscopy (EGD) with biopsy

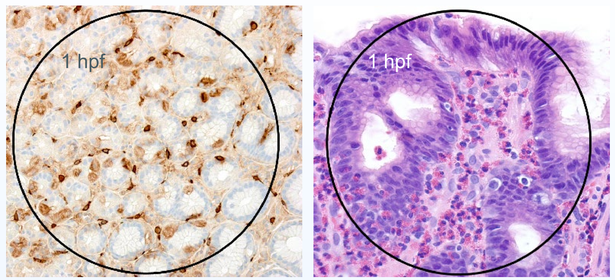
EGD with Biopsy

- Multiple biopsies were taken from each symptomatic subject according to a standardized protocol:
  - 8-10 gastric biopsies
  - 4-6 duodenal biopsies
  - 4-6 esophageal biopsies (only if subject had a history of EoE or if EoE features were observed during EGD)

Histologic Criteria

- Single pathologist evaluated stained biopsy samples and counted eosinophils
- Entry criteria
  - **≥30 eos/hpf in 5 hpfs (stomach)** and/or **≥30 eos/hpf in 3 hpfs (duodenum)**
  - No other known cause for GI symptoms or tissue eosinophilia

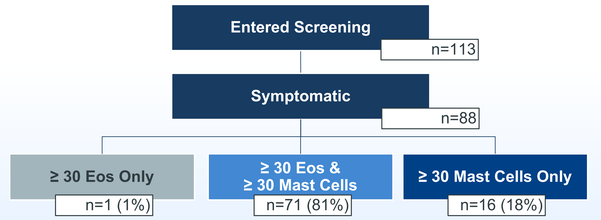
Figure 4. Counting Eosinophils and Mast Cells



- Eosinophils and mast cells were identified by H&E and immunohistochemistry (IHC), respectively. IHC was performed by staining with a mouse anti-human mast cell tryptase primary antibody (Clone AA1) followed by incubation with a peroxidase-labelled anti-mouse polymer secondary antibody and then counterstained with hematoxylin.
- Mast cells and eosinophils were counted in 5 separate non-overlapping high-power fields (hpf; area of 0.237 mm^2) for each biopsy sample
- The patients qualified for study if the peak counts in 5 hpfs in the stomach (or 3 hpf in the duodenum) was ≥30 eos/hpf
- The patients were determined to have elevated mast cells if the average of the peak counts in 5 hpfs in the stomach (or 3 hpfs in the duodenum) was ≥30 mast cells/hpf (normal mast cell counts from literature 12-20 /hpf[4-8])

RESULTS

Figure 5. Patient Distribution



- 87 of 88 symptomatic patients had elevated mast cell counts

Table 1. Baseline Characteristics

	≥30 Eos; ≥30 MC[a] (n=72)	<30 Eos; ≥30 MC (n=16)
Age, Median Years (Range)	42 (18-74)	50 (19-78)
Female, n (%)	43 (60%)	11 (69%)
Race - White, n (%)	66 (92%)	14 (88%)
History of Atopy/Allergy[b], n (%)	56 (78%)	4 (25%)
Blood Eos (cells/μL), Mean	662	107
Steroid Use n, (%)	16 (22%)	2 (13%)

a One patient with mast cells below 30 included
b Medical history at screening of asthma, rhinitis, food allergy, atopic dermatitis, seasonal allergy, environmental allergy, or pollen allergy

Figure 6. Mast Cells Are Consistently Elevated in Stomach and Duodenal Biopsies in Clinically Suspected EGID Even in Absence of Tissue Eosinophilia

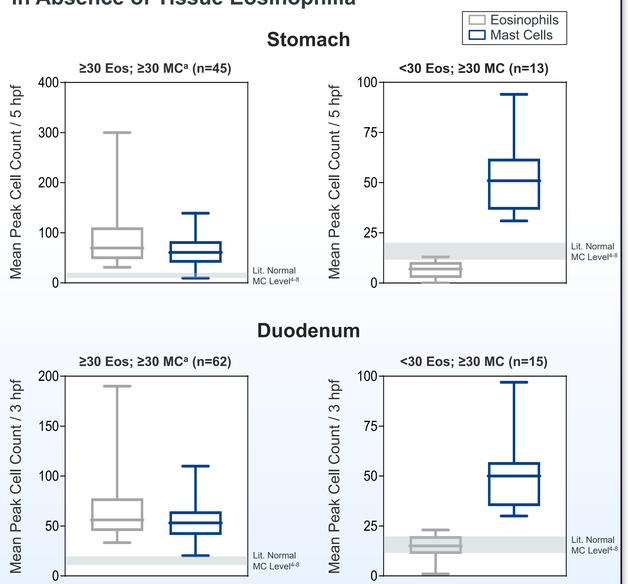
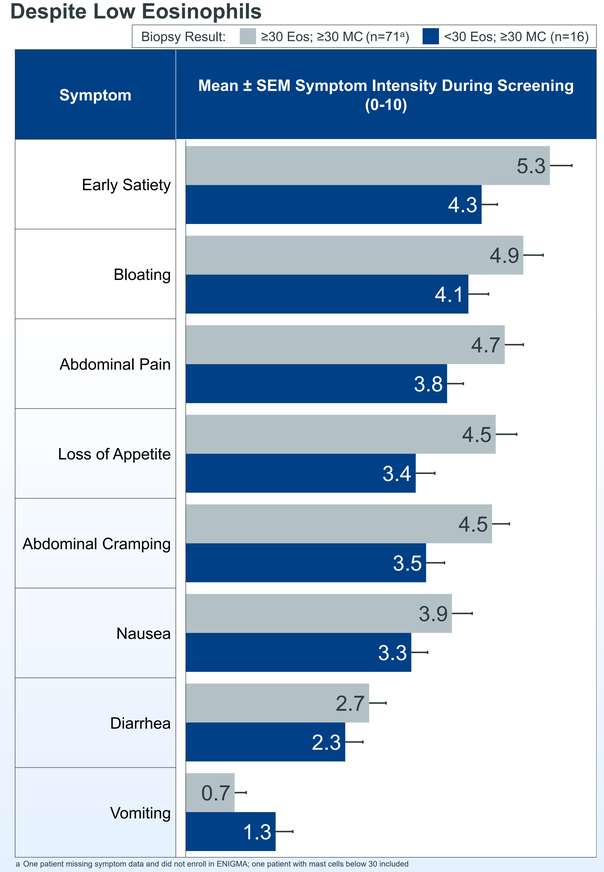


Figure 6. Data are plotted as box and whisker plots of peak mean cell count/hpf. The box plot represents the 25th and 75th percentiles, the internal horizontal line indicates the median and the T-bars indicate the minimum and maximum counts. The shaded gray bar indicates normal mast cell counts in the stomach & small intestine.[4-8]

a One patient with mast cells below 30 included

Figure 7. Similar Symptom Profiles Between Groups Despite Low Eosinophils



a One patient missing symptom data and did not enroll in ENIGMA; one patient with mast cells below 30 included

CONCLUSIONS/DISCUSSION

- Eighty-eight patients with suspected EG and/or EGE and active symptoms underwent endoscopy and biopsy: 72 of 88 met histologic eosinophil criteria for the study
- 87 of 88 (99%) patients screened had elevated mast cell counts in gastric and/or duodenal tissue biopsies
- Symptom profiles were similar between patients with and without tissue eosinophilia
- These data suggest that mast cells play an important pathogenic role in patients with suspected EG/EGE and raise the possibility of a non-eosinophilic condition driven by mast cells
- Due to lirentelimab's ability to inhibit mast cells, patients with elevated mast cells without tissue eosinophilia were offered to participate in an open label lirentelimab clinical trial (Data expected in 2020)

This study was funded by Allakos, Inc. and in part by Division of Intramural Research, NIAID, NIH

References:
1) Caldwell et al. J Allergy Clin Immunol. 2014.
2) Youngblood et al. JCI Insight. 2019
3) Jensen ET, Martin CF, Kappelman MD, Dellon ES. J Pediatr Gastroenterol Nutr. 2016 Jan;62(1):36-42.
4) Hahn et al., Am J Surg Pathol. 2007 Nov;31(11):1669-76
5) Tison et al., J Allergy Clin Immun. 2010 Feb;125(2):AB182
6) Walker et al., Aliment Pharmacol Ther. 2009 Apr 1;29(7):765-73
7) Martinez et al., Gut. 2013 Aug;62(8):1160-8
8) Doyle et al., Am J Surg Pathol. 2014 Jun;38(6):832-43

Presented at the American College of Gastroenterology, San Antonio, TX October 25th-30th 2019