Lirentelimab (AK002), an Anti-Siglec-8 Antibody, Inhibits IL-33-mediated Mast Cell Activation and Neutrophilic Inflammation

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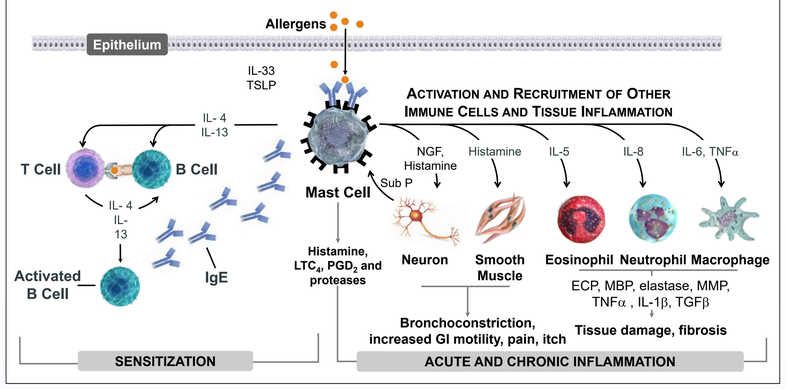
BACKGROUND

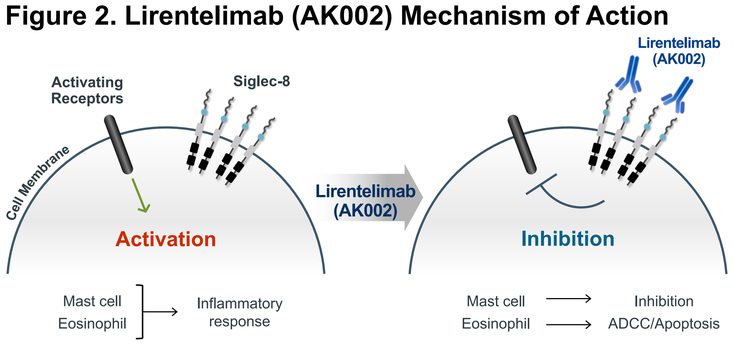
- IL-33 stimulation of mast cells has been shown to enhance IgE-mediated degranulation and promote both allergic and non-allergic inflammation
- Siglec-8 targeting monoclonal antibodies (mAb) have previously been shown to inhibit IgE-mediated mast cell activation and deplete eosinophils
- However, the effect of a Siglec-8 mAb has not been evaluated in IL-33-driven models of inflammation

Figure 1. Mast Cells and Eosinophils are Key Drivers of Acute and Chronic Inflammation



- Siglec-8 is an inhibitory receptor selectively expressed on human eosinophils and mast cells, and therefore represents a novel target for the treatment of debilitating allergic, inflammatory, and proliferative diseases
- 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 blood eosinophils and apoptosis of tissue eosinophils
  - Inhibition of mast cells
- Here we evaluate the inhibitory activity of lirentelimab (AK002) in a mast cell-mediated, IL-33-driven inflammation mouse model

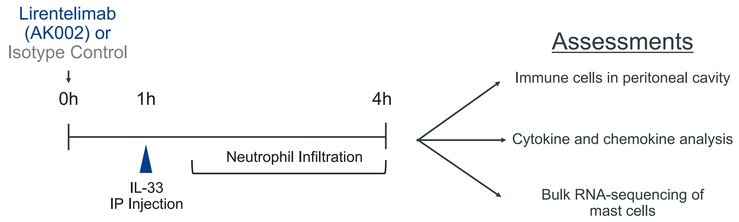
Figure 2. Lirentelimab (AK002) Mechanism of Action



METHODS

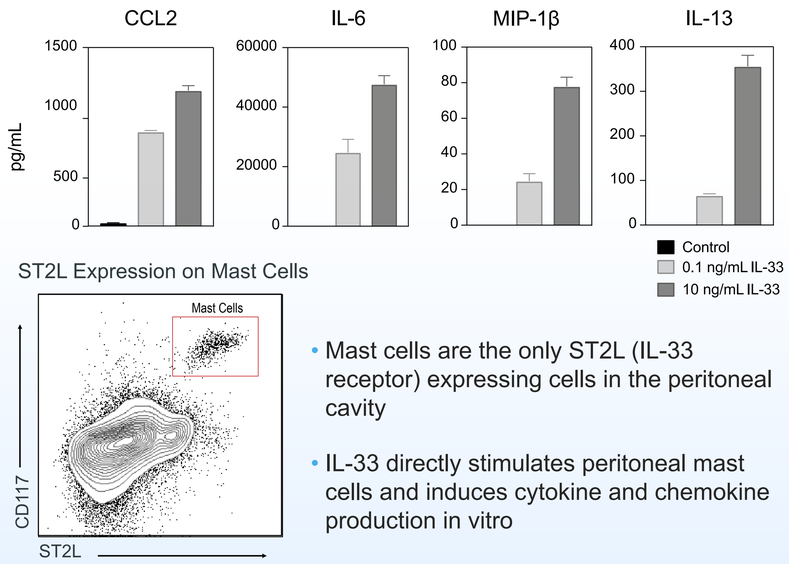
- Acute neutrophil infiltration was induced by intraperitoneal injection of IL-33 (50ng) in Siglec-8 transgenic mice[1]
- Immune cells in the peritoneal cavity were harvested by lavage and analyzed by flow cytometry; cytokines and chemokines were quantified in the peritoneal lavage by MSD
- Mast cells from individual mice were isolated by FACS and subjected to RNA-sequencing using the Illumina HiSeq platform
- RNAseq data was aligned to the mouse GRCm38.p6 r98 reference; counts were determined using Rsubread; fold change and differential expression between treatment groups were computed using DESeq2

Figure 3. Mouse Model of IL-33-Induced Neutrophil Infiltration[1]



RESULTS

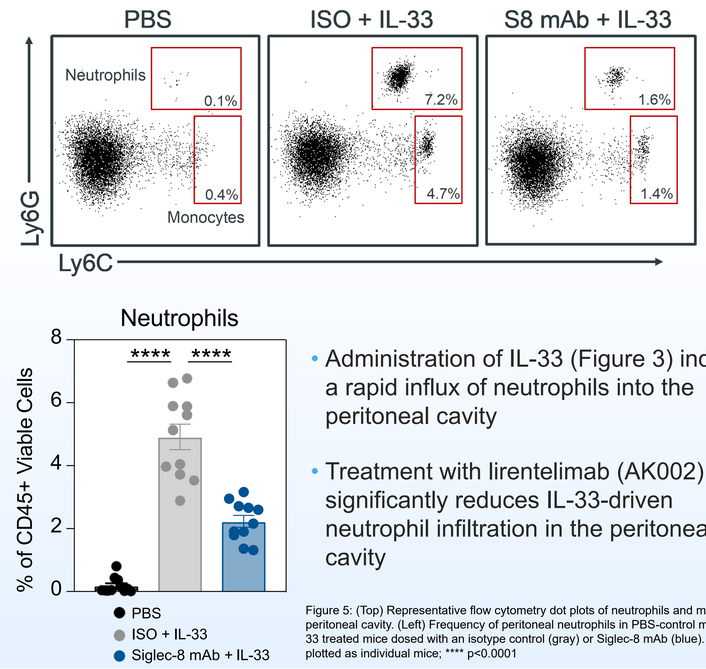
Figure 4. IL-33 Directly Induces Cytokine Production from Peritoneal Mast Cells in vitro



- Mast cells are the only ST2L (IL-33 receptor) expressing cells in the peritoneal cavity
- IL-33 directly stimulates peritoneal mast cells and induces cytokine and chemokine production in vitro

Figure 4: (Top) Cytokine and chemokine levels (pg/mL) in the cell-free supernatant of cultured peritoneal mast cells after overnight stimulation with 0.1 ng/mL mIL-33 (light gray), 10 ng/mL mIL-33 (dark gray) or unstimulated control (black). (Bottom) Expression of ST2L on mast cells in freshly isolated peritoneal lavage; mast cells are gated on CD45+ 7AAD- cells.

Figure 5. Lirentelimab (AK002) Reduces IL-33-driven Neutrophil Infiltration



- Administration of IL-33 (Figure 3) induces a rapid influx of neutrophils into the peritoneal cavity
- Treatment with lirentelimab (AK002) significantly reduces IL-33-driven neutrophil infiltration in the peritoneal cavity

Figure 5: (Top) Representative flow cytometry dot plots of neutrophils and monocytes in the peritoneal cavity. (Left) Frequency of peritoneal neutrophils in PBS-control mice (black) or IL-33 treated mice dosed with an isotype control (gray) or Siglec-8 mAb (blue). Graphs are plotted as individual mice; **** p<0.0001

Figure 6. Lirentelimab (AK002) Decreases Monocyte and Eosinophil Infiltration Induced by IL-33

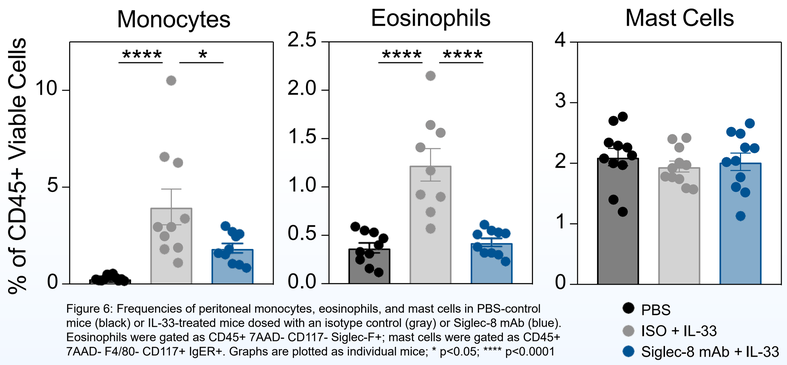


Figure 6: Frequencies of peritoneal monocytes, eosinophils, and mast cells in PBS-control mice (black) or IL-33-treated mice dosed with an isotype control (gray) or Siglec-8 mAb (blue). Eosinophils were gated as CD45+ 7AAD- CD117- Siglec-F+; mast cells were gated as CD45+ 7AAD- F4/80- CD117+ IgER+. Graphs are plotted as individual mice; * p<0.05; **** p<0.0001

- IL-33 administration also drives infiltration of monocytes and eosinophils into the peritoneal cavity that is reduced by lirentelimab (AK002)
- Consistent with previous findings, lirentelimab (AK002) does not reduce mast cell numbers, suggesting the reduction in IL-33-mediated leukocyte infiltration is most likely mast cell inhibition

Figure 7. Lirentelimab (AK002) Decreases IL-33-induced Cytokines and Chemokines in vivo

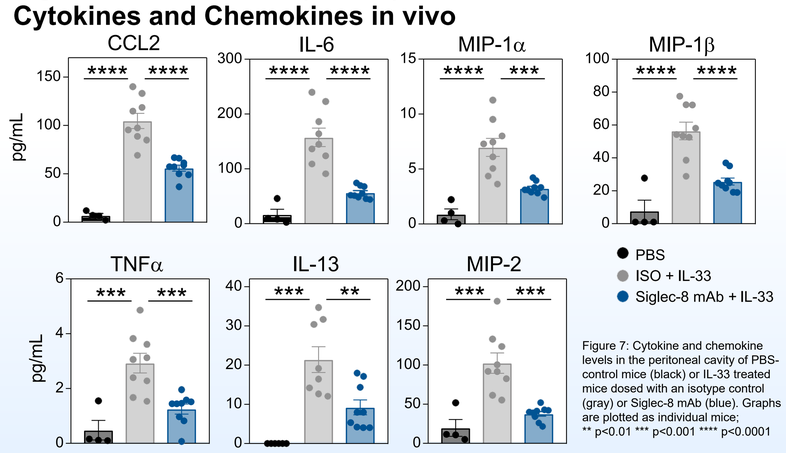


Figure 7: Cytokine and chemokine levels in the peritoneal cavity of PBS-control mice (black) or IL-33 treated mice dosed with an isotype control (gray) or Siglec-8 mAb (blue). Graphs are plotted as individual mice; ** p<0.01 *** p<0.001 **** p<0.0001

Figure 8. Lirentelimab (AK002) Globally Inhibits IL-33 Activated Mast Cells

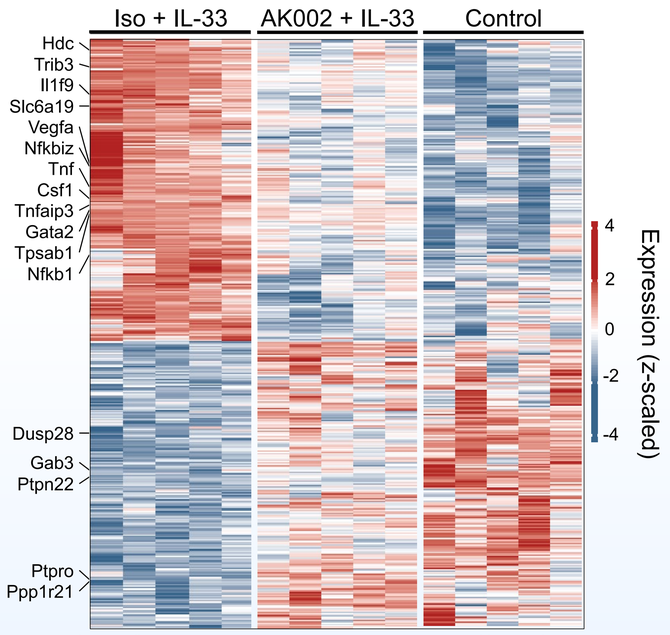


Figure 8: Heatmap of the top differentially expressed genes in peritoneal mast cells that were FACS-sorted after treatment with IL-33 + isotype mAb (left), IL-33+ Siglec-8 mAb (middle), or PBS-control (right) and subjected to RNA-sequencing. Genes displayed in the heatmap were the top 445 genes that were significantly modulated by IL-33 and normalized by AK002 treatment. Each column represents transcriptome data from individual mice (n=5/group)

- IL-33 in vivo administration induces global changes in the transcriptome of peritoneal mast cells that is modulated by lirentelimab (AK002)
- These data strongly suggest that lirentelimab (AK002) globally inhibits IL-33-mediated mast cell activation at the transcriptome level

Figure 9. Lirentelimab (AK002) Inhibits Downstream Signaling Pathways of IL-33 Activation

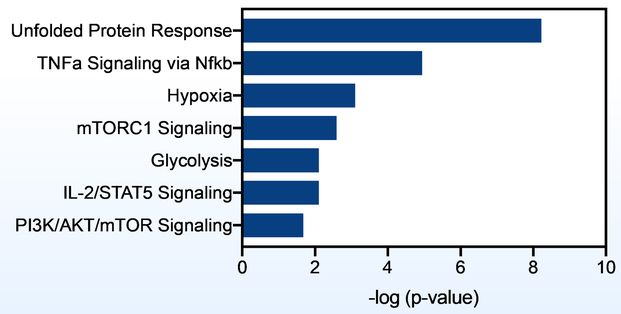


Figure 9: Hallmark gene set-enrichment analysis (GSEA) of genes upregulated with IL-33 treatment and downregulated with AK002 as shown above. Genes were selected based on criteria of L2F > 0.4 in Isotype + IL-33 and < -0.4 in IL-33 + AK002.

CONCLUSIONS/DISCUSSION

- Lirentelimab (AK002) significantly reduces IL-33-driven inflammation by directly inhibiting IL-33-mediated mast cell activation
- Targeting Siglec-8 may have the potential to treat diseases associated with mast cells and eosinophils, including those where IL-33 can exacerbate immune responses, such as eosinophilic gastrointestinal diseases, atopic dermatitis, asthma, and food allergy

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Reference: 1) Model adapted from: Enoksson, M., et al. Blood 2013.