Atopic Dermatitis Skin Biopsies Have High Numbers of Activated Mast Cells that Are Inhibited by Lirentelimab (AK002) After Stimulation Ex Vivo

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BACKGROUND

- Loss of epithelial barrier integrity is a critical step in the development of atopic dermatitis (AD) whereby the alarmin cytokines IL-33 and TSLP activate inflammatory cells such as mast cells (MCs) (Figure 1)
- While MCs have been shown to be elevated AD, there is need for further characterization of their pathogenic role
- Siglec-8 is an inhibitory receptor expressed on mast cells and eosinophils and represents a new potential therapeutic target for AD given the pathogenic role of MCs

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

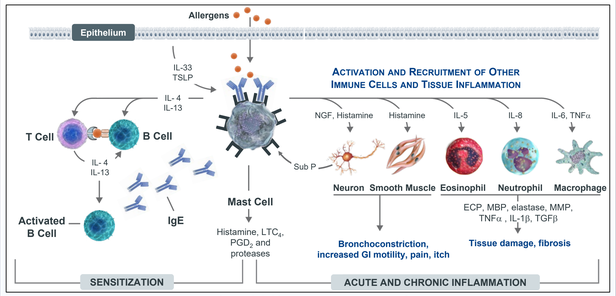
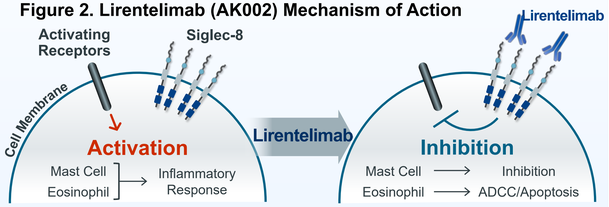


Figure 2. Lirentelimab (AK002) Mechanism of Action



- Siglec-8 is an inhibitory receptor selectively expressed on human eosinophils and mast cells, and therefore represents a novel target for the treatment of debilitating allergic, inflammatory, and proliferative diseases
- Lirentelimab 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 immunophenotype mast cells and examine the ex vivo activity of Lirentelimab in AD biopsies

METHODS

- Single-cell suspensions were prepared by enzymatic & mechanical digestion of fresh biopsies from patients clinically diagnosed with AD (n=6) or non-disease control skin tissue (n=10)
- 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 AD biopsies or non-diseased skin tissues followed by overnight incubation with or without PMA/Ionomycin
- Cell-free supernatants 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, INFɣ, TNFα, CCL2, CCL3, CCL4, and VEGF

Figure 3. Study Design

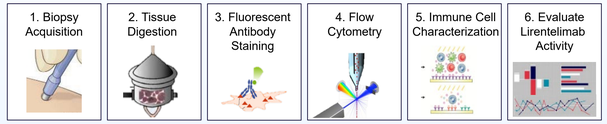
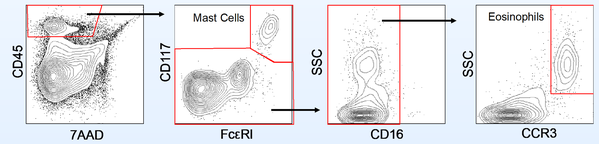


Figure 4. Flow Cytometry Gating Strategy for Mast Cells and Eosinophils in AD Biopsy Tissue



RESULTS

Figure 5. Non-Diseased Human Skin Mast Cells Express Siglec-8 and Multiple Types of Activating Receptors

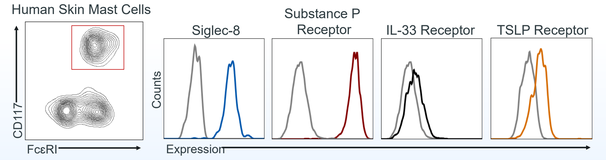


Figure 5: (Left) Representative contour plot of human mast cells from skin tissue. (Right) Expression of Siglec-8 (blue), Substance P receptor (red), ST2L (black), and TSLP receptor (yellow) on human skin mast cells compared to a fluorescence minus one (FMO) control (gray).

Figure 6. Lirentelimab (AK002) Inhibits IgE-Dependent Human Skin Mast Cell Activation

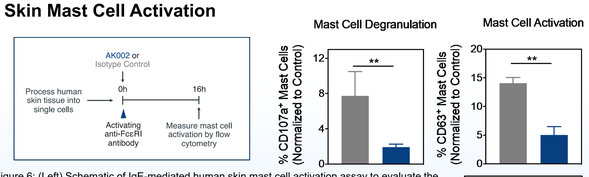


Figure 6: (Left) Schematic of IgE-mediated human skin mast cell activation assay to evaluate the inhibitory activity of AK002. (Right) Markers of mast cell degranulation (CD107a) and activation (CD63) as assessed by flow cytometry for human skin mast cells. Activation was normalized to an unstimulated control. Data are shown as mean +/- SEM for n=5 donors; * p<0.05; ** p<0.01

- AK002 inhibits FcεRI activation of skin mast cells, suggesting an anti-Siglec-8 approach may be effective in MC-driven skin diseases, such as AD

Figure 7. Mast Cells and Eosinophils are Elevated in AD Skin Biopsies

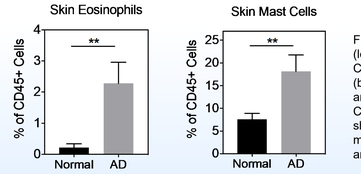


Figure 7: Percentage of eosinophils (left) and mast cells (right) among CD45+ immune cells in non-diseased (black) or AD (gray) skin. Eosinophils and mast cells were gated on viable CD45+ cells in processed skin tissue as shown in figure 4. Data are shown as mean +/- SEM for n=10 non-diseased and n=6 for AD donors; ** p<0.01

Figure 8. Resting Mast Cells are Activated in AD Skin Biopsies

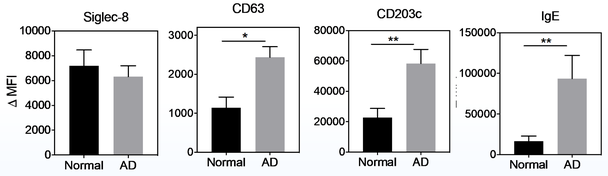


Figure 8: The expression of Siglec-8 and the known mast cell activation markers, CD63, CD203c, and IgE were determined by flow cytometry in non-diseased normal tissue (black) and AD skin tissue (gray). Data are shown as mean +/- SEM for n=10 non-diseased and n=6 for AD donors; ** p<0.01 * p<0.05; *** p<0.001

- Siglec-8 expression remains high on human skin mast cells independent of disease state
- Resting mast cells in AD skin tissue display an activated atopic phenotype compared to non-diseased skin mast cells

Figure 9. Lirentelimab Inhibits IL-33/TSLP-mediated MC Activation in AD Skin Biopsies

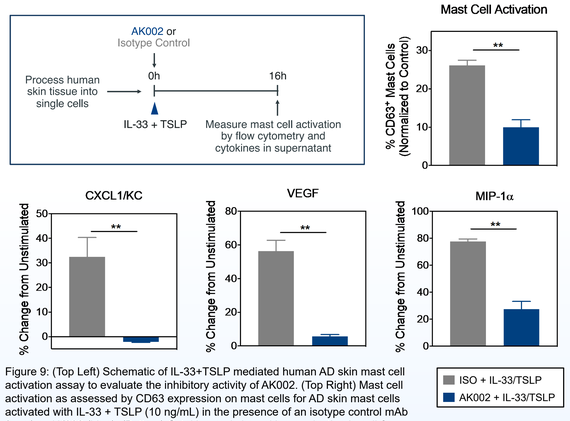


Figure 9: (Top Left) Schematic of IL-33+TSLP mediated human AD skin mast cell activation assay to evaluate the inhibitory activity of AK002. (Top Right) Mast cell activation as assessed by CD63 expression on mast cells for AD skin mast cells activated with IL-33 + TSLP (10 ng/mL) in the presence of an isotype control mAb (gray) or AK002 (blue). (Bottom) Cytokine and chemokine production in cell-free supernatant induced by IL-33 + TSLP from AD skin mast cells treated with an isotype control mAb (gray) or AK002 (blue). Data are shown as mean +/- SEM for n=3 AD donors; ** p<0.01

- Mast cells in AD skin biopsies are activated by IL-33/TSLP suggesting they are important target cells for alarmin cytokines released by epithelial cells
- Treatment with AK002 significantly reduces IL-33/TSLP mast cell activation as evidenced by decreased surface markers of activation and cytokine production

CONCLUSIONS

- Human skin mast cells express the inhibitory receptor Siglec-8, and activation of mast cells cells via FcεRI is inhibited with lirentelimab
- Mast cells are elevated in number and are basally activated in AD biopsies with high levels of surface-bound IgE
- Lirentelimab inhibits IL-33/TSLP-mediated mast cell activation in AD skin biopsies, suggesting lirentelimab can broadly inhibit multiple modes of mast cell stimulation including, IgE, IL-33, and TSLP
- Mast cells appear to be important in AD, and targeting mast cells via Siglec-8 with lirentelimab may represent a novel therapeutic approach to the treatment of AD and other allergic diseases

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