Poster 518

An Agonistic Monoclonal Antibody Against Siglec-6 Broadly Inhibits Mast Cell Activation in Transgenic Mice

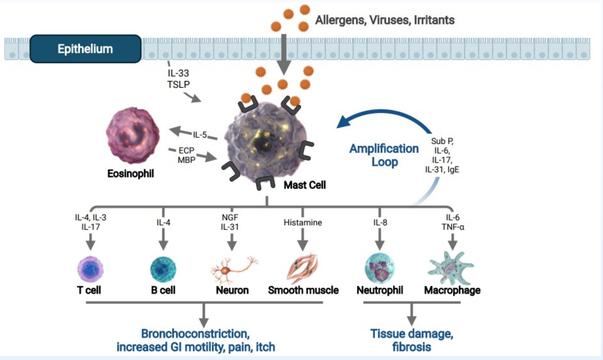
Zachary Benet[1], Thuy Luu[1], Emily C. Brock[1], Robert Sanchez[1], Lisa M. McEwen[2], Krysta Coyle[2], Katherine Chang[1], John Leung[1], Wouter Korver[1], Julia Schanin[1] and Bradford A. Youngblood[1]

[1]Allakos Inc., San Carlos, CA; [2]LM Biostat Consulting Inc., British Columbia, Canada

BACKGROUND

- Mast cells (MCs) regulate chronic inflammation through a myriad of activating cell surface receptors[1]
- Dysregulation of MC activation through IgE-dependent and -independent mechanisms (i.e. cytokines, neuropeptides) contributes to allergic and non-allergic diseases[2]
- MC-targeting strategies have focused on neutralizing individual mediators or activating receptors which may be insufficient to broadly reduce MC activity[3]
- Molecules that dampen multiple pathways of MC activation, such as sialic acid-binding Ig-like lectins (Siglecs), represent novel therapeutic options for inflammatory diseases[4]
- Siglec-6 is an inhibitory receptor selectively expressed on human mast cells, and represents a novel target for the treatment of debilitating allergic, inflammatory, and proliferative diseases[5]
- AK006 is a humanized, agonistic Siglec-6 antibody that induces broad MC inhibition and reduces MCs via ADCP
- Because MCs contribute to disease through multiple activating pathways, we evaluated the activity of an agonistic Siglec-6 monoclonal antibody (mAb) in models of IgE-dependent and -independent MC activation

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

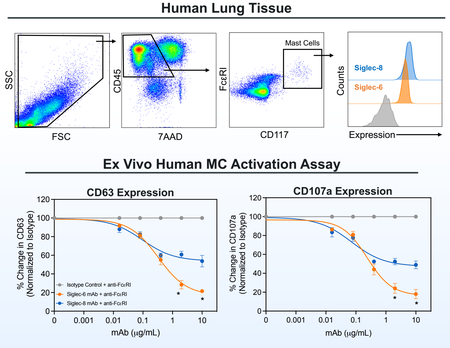


METHODS

- Siglec-6-Tg (S6-Tg) mice were generated in C57BL/6 mice and constitutively express human Siglec-6 on MCs
- Allergic enteritis was induced through sensitization and intragastric with OVA in S6-Tg mice
- IL-33-driven skin inflammation was induced by intradermal injection of 250ng of recombinant mouse IL-33
- Mrgprb2/MRGPRX2-driven rosacea model was induced by intradermal injection of LL-37 in S6-Tg mice
- To evaluate the activity of targeting Siglec-6, mice were intravenously injected with a Siglec-6 mAb or an isotype-matched control mAb
- Cytokines and soluble mediators were measured by MSD or ELISA

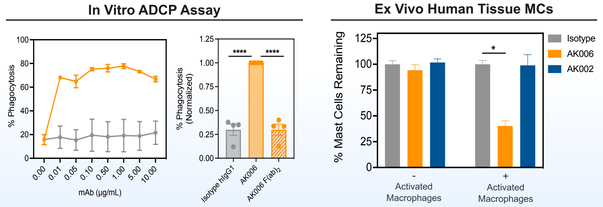
RESULTS

Figure 2. AK006 Induces Deeper MC Inhibition than AK002



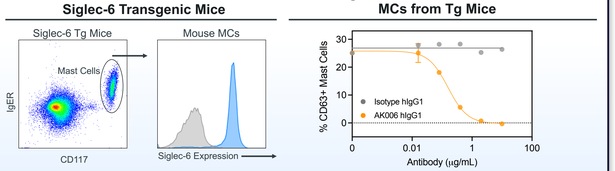
(Top) MC gating strategy and Siglec-6/8 expression in human lung tissue. (Bottom) Percentage of CD63 or CD107a positive lung tissue MCs upon titration of AK006 (orange), AK002 (blue), or isotype control (gray) antibodies in combination with anti-FcεRI-mediated MC activation. * = p <0.05 (n=3 donors)

Figure 3. AK006 Reduces MC Numbers via ADCP



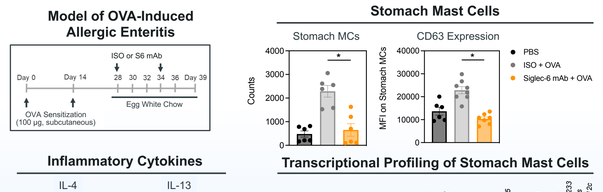
(Left) Percentage of phagocytosis of MCs by macrophages in the presence of titrating concentrations of AK006 (solid orange), AK006 F(ab)$_2$ (hashed orange, or isotype control (gray). (Right) Percentage of human lung tissue MCs remaining after overnight incubation with isotype control (gray), AK006 (orange), or AK002 (blue) in the presence or absence of activated human macrophages. . * = p <0.05, *** = p<0.001

Figure 4. Siglec-6 Tg Mice Express Functional Receptor on Mouse MCs



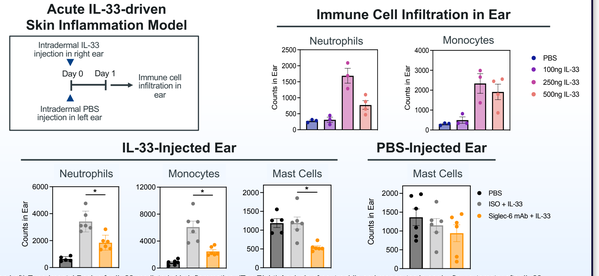
(Left) Representative dot plots of peritoneal lavage from Siglec-6 transgenic mice and Siglec-6 expression on peritoneal MCs (blue). (Right) Expression of CD63+ MCs as measured by flow cytometry after cross-linking FcεRI and treated with either isotype (gray) or AK006 (orange) antibodies.

Figure 5. Siglec-6 mAb Reduces Allergic Enteritis via MC Inhibition



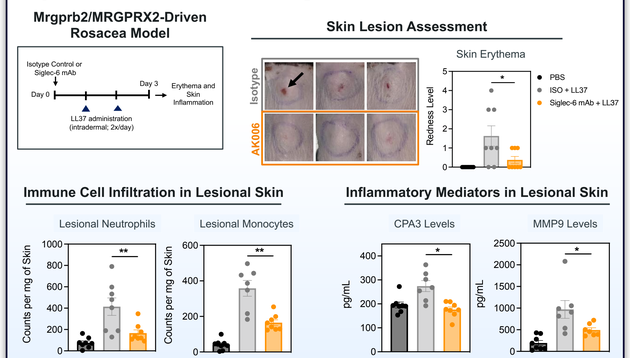
(Top left) Experimental Design for Allergic Enteritis. (Top Right) Flow cytometry analysis of MC numbers and CD63 expression on MCs isolated from the stomach epithelium. (Bottom Left) Serum levels of Type 2 cytokines IL-4 and IL-13 at study takedown measured by MSD. (Bottom Right) Differentially expressed genes from sorted stomach MCs analyzed by RNA-seq at study takedown that were significantly induced in isotype control-treated mice. . * = p <0.01.

Figure 6. Siglec-6 mAb Inhibits IL-33-Driven Skin Inflammation



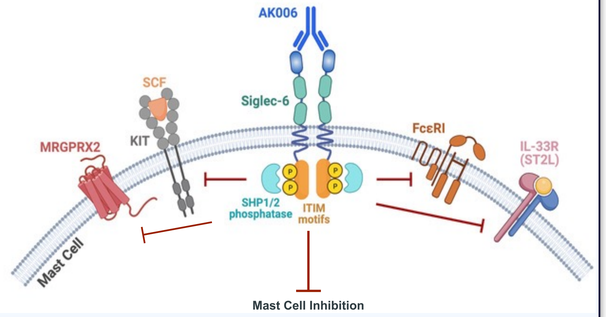
(Top Left) Experimental Design for IL-33-mediated skin inflammation. (Top Right) Analysis of neutrophils and monocytes in ear by flow cytometry after IL-33 administration at different concentrations. (Bottom Left) Counts of neutrophils, monocytes, and MCs in IL-33 (250ng) injected ears via flow cytometry in mice that received isotype control or Siglec-6 mAb 24 hours before IL-33 challenge. (Bottom Right) Numbers of MCs in PBS injected ears. * = p <0.05.

Figure 7. Mrgprb2/MRGPRX2-Induced Skin Inflammation and Erythema Are Reduced with a Siglec-6 mAb



(Top Left) Mrgprb2-driven LL-37 rosacea skin model experimental schematic. (Top Right) Pictures of skin lesions and erythema scoring in LL-37-driven inflammation model. (Bottom Left) Counts of neutrophils and monocytes in LL-37 injected skin lesions. (Bottom Right) CPA3 and MMP9 levels from the supernatants of ex vivo cultured skin lesions for 3 hours in media. * = p <0.05, ** = p < 0.001.

CONCLUSIONS



- AK006 displays differential MC inhibitory and ADCP activity compared to AK002
- Targeting Siglec-6 with an agonist mAb significantly suppressed allergic and non-allergic models of inflammation via broad MC inhibition and local reduction of MCs
- These findings support AK006 as a potential therapeutic across multiple MC-driven diseases

References: 1. Theoharides T, et al. Biochim Biophys Acta 2012; 2. Paivandy A and Pejler G. J Innate Immun 2021; 3. Dahlin JA, et al. Allergy 2012; 4. Macauley MS, et al. Nat Rev Immunol 2014; 5. Schanin J, et al. Comm Bio 2022

Study funded by Allakos Inc. Presented at the American Academy of Allergy Asthma & Immunology (AAAAI) Annual Meeting Feb 24-27, 2023