P#1104

AK002, a Novel Humanized Monoclonal Antibody to Siglec-8, Inhibits Mast Cell Activity and Depletes Eosinophils in Ex Vivo Bone Marrow Tissue from Patients with Systemic Mastocytosis

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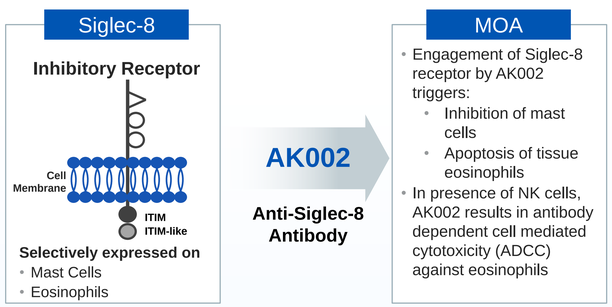
BACKGROUND

- Systemic Mastocytosis (SM) is a rare disease characterized by the clonal proliferation and accumulation of mast cells in the bone marrow, respiratory and gastrointestinal tracts, and organs such as the skin, liver, spleen, and brain
- Common symptoms include pruritus, flushing, headache, cognitive impairment, fatigue, diarrhea, abdominal pain, hypotension and skin lesions, as well as an increased risk for osteoporosis and anaphylaxis
- SM is currently managed with antihistamines, cromolyn sodium, and leukotriene blocking agents, which lack efficacy in many patients
- In addition, glucocorticoids can provide temporary relief in some cases; however long-term treatment with steroids is not appropriate due to their many side effects

Figure 1. AK002 Mechanism of Action



- Siglec-8 is an inhibitory receptor selectively expressed on human mast cells and eosinophils, representing a novel target for the treatment of SM
- Antibodies to Siglec-8 have been shown to broadly inhibit mast cell activity and induce apoptosis of eosinophils
- AK002 is a novel, humanized, non-fucosylated IgG1 monoclonal antibody to Siglec-8
- This study evaluates the expression of Siglec-8 and ex vivo activity of AK002 on mast cells and eosinophils in bone marrow aspirates from patients with SM

Figure 2. AK002-001 Indolent Systemic Mastocytosis Phase 1 Study

Design	Key Endpoints		Status
• Open-label, pilot study • AK002 Single-Dose Phase: Single doses of 0.0003, 0.001, 0.003, 0.01, 0.03, 0.1, 0.3, or 1.0 mg/kg • AK002 Multiple-Dose Phase: 6 monthly doses every 4 weeks. 1.0 mg/kg starting dose followed by either 1, 3, 6, or 10 mg/kg	**Primary**	• Safety and tolerability	• Enrollment and dosing complete • 25 patients enrolled (13 in single-dose, 12 in multiple-dose)
	Secondary	• Patient reported outcomes: itching, skin flushing, diarrhea, abdominal pain, fatigue, headache	

METHODS

- Bone marrow aspirates and blood were obtained from patients clinically diagnosed with SM; heathy subject blood was obtained from Stanford Blood Center (Palo Alto, CA). Aspirates and blood were processed to remove red blood cells before use
- Multi-color flow cytometry was used to quantify immune cells and analyze surface markers
- Bone marrow aspirates with removed red blood cells were cultured in RPMI + 10% FBS overnight with either 1 µg/mL isotype control mAb (afucosylated hIgG1) or AK002 followed by flow cytometry or Luminex analysis

Table 1: SM Patient Characteristics

Patient Number	Diagnosis	Current Therapy	Biospecimens Collected
AL01	SM-CMML	None	BM Aspirate/Peripheral Blood
AL02	SM-MDS	High dose steroids	BM Aspirate
AL03	SM-CEL	Midostaurin	BM Aspirate/Peripheral Blood
AL04	ASM	Midostaurin	BM Aspirate/Peripheral Blood
AL05	SSM	None	BM Aspirate/Peripheral Blood
AL06	SM-CMML	Ibrutinib	Peripheral Blood
AL07	SM-CMML	Cladribine	BM Aspirate/Peripheral Blood
AL08	SM-CMML	Unknown	BM Aspirate/Peripheral Blood
AL09	ASM	Midostaurin	BM Aspirate
AL10	ISM	None	BM Aspirate
AL11	ASM	Midostaurin	BM Aspirate
AL12	ASM	Midostaurin	BM Aspirate

SM, systemic mastocytosis; CMML, chronic myelomonocytic leukemia; MDS, myelodysplastic syndrome; CEL, chronic eosinophilic leukemia; ASM, aggressive systemic mastocytosis; ISM, indolent systemic mastocytosis

RESULTS

Figure 3: SM Patient Bone Marrow Mast Cells Express Siglec-8 and Display a Diseased Phenotype

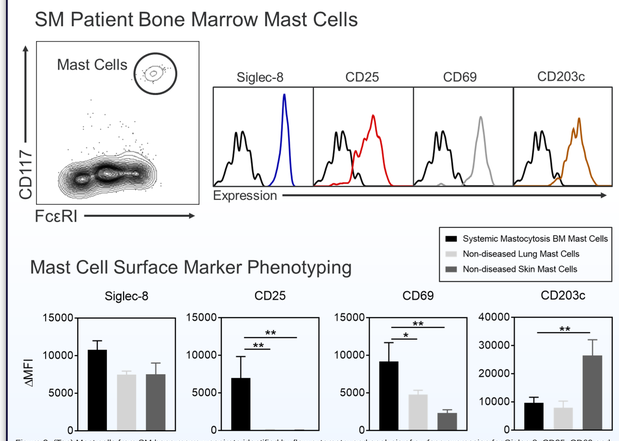


Figure 3: (Top) Mast cells from SM bone marrow aspirate identified by flow cytometry and analysis of surface expression for Siglec-8, CD25, CD69 and CD203c compared to a fluorescence minus one (FMO) control (black). (Bottom) Expression of surface markers on mast cells from SM patient bone marrow (black), non-diseased lung tissue (light gray), or non-diseased skin tissue (dark gray) analyzed by flow cytometry. Mast cells were gated on viable, CD45+, CD117+, FcεRI+ for n=6 SM patients, n=9 lung and skin tissue donors. ΔMFI was calculated by subtracting MFI from a negative FMO control. * p<0.05; **p<0.01

- Siglec-8 is highly expressed on all bone marrow mast cells from SM patients and CD25 and CD69 expression is significantly elevated on SM bone marrow mast cells compared to non-diseased lung and skin tissue mast cells
- Primary skin mast cells have significantly higher expression of CD203c

Figure 4. AK002 Significantly Reduced Eosinophils in ex vivo SM Patient Bone Marrow

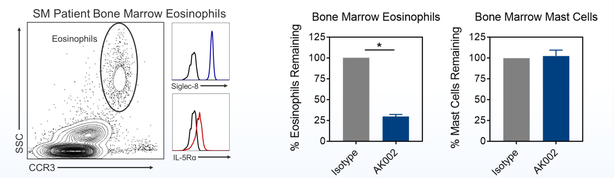


Figure 6: (Left) Eosinophils from SM patient bone marrow aspirate identified by flow cytometry and analysis of surface expression for Siglec-8 and IL-5Rα compared to a fluorescence minus one (FMO) control (black). (Right) Bone marrow aspirate from SM patients was cultured overnight with either an isotype control mAb (gray) or AK002 (blue) and eosinophils or mast cells were quantified the following day by flow cytometry. Eosinophils were gated on viable, CD45+, CD117-, CD16- SSC^{lo}, CCR3+; Mast cells were gated on viable, CD45+, CD117+, FcεRI+; Data shown are mean +/- SD from 3 SM patients; *p<0.05

- Siglec-8 is highly expressed on mature bone marrow eosinophils from SM patients
- AK002 significantly reduced ex vivo bone marrow eosinophils, but not mast cells, from SM patients consistent with previous experiments

Figure 5. AK002 Reduced the Expression of CD25 and CD69 on ex vivo Bone Marrow Mast Cells from SM Patients

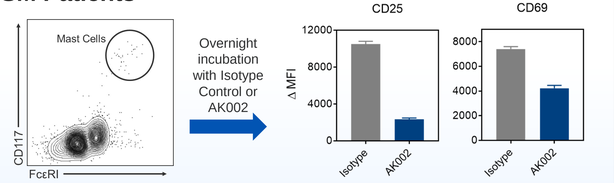


Figure 4: (Left) Mast cells from SM patient bone marrow were identified by flow cytometry. (Right) Bone marrow aspirate from SM patients was cultured overnight with either an isotype control mAb (gray) or AK002 (blue) and the expression of CD25 and CD69 on mast cells was analyzed the following day by flow cytometry. Mast cells were gated on viable, CD45+, CD117+, FcεRI+; ΔMFI was calculated by subtracting MFI from a negative FMO control. Data shown is ΔMFI +/- SD from 1 SM patient that is representative of 4 SM patients.

- Overnight treatment with AK002 reduced the expression of the SM-markers, CD25 and CD69 on bone marrow mast cells from SM patients

Figure 6. AK002 Reduced the Level of Mast Cell-Associated Mediators Produced in Supernatant of Cultured Bone Marrow Cells

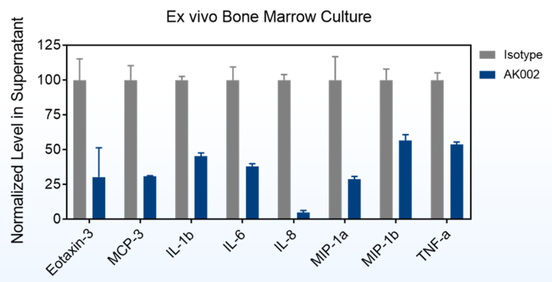


Figure 5: SM patient bone marrow aspirate was cultured overnight with either an isotype control mAb (gray) or AK002 (blue). The next day cytokines and chemokines in the supernatant were analyzed using Luminex (Millipore). The percentage of cytokine in the supernatant was calculated by normalizing the level of mediator in the isotype control to 100 percent. All mediators shown were above the limit of detection. Data shown is mean +/- SD from 1 SM patient. This data is representative of 3 SM patients.

- AK002 reduced the level of mast cell-associated mediators produced in ex vivo bone marrow supernatants from SM patients, suggesting inhibition of diseased mast cells

Figure 7. SM Patients Display Elevated Levels of Serum Cytokines and Chemokines Compared to Healthy Subjects

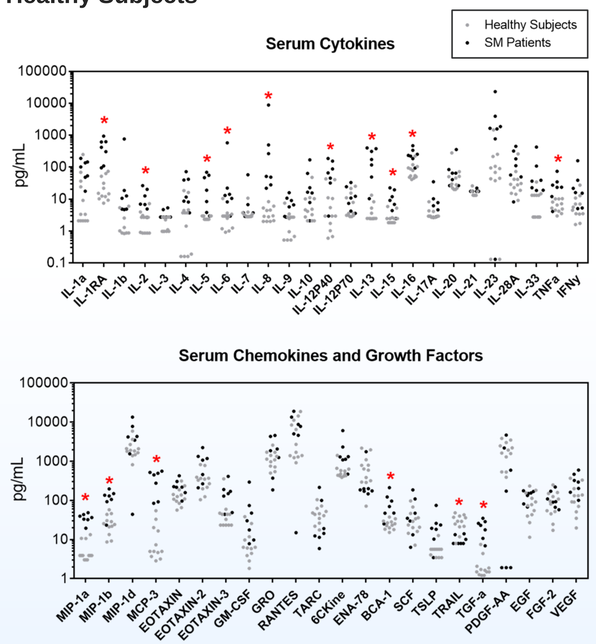


Figure 7: Level of (top) cytokines or (bottom) chemokines and growth factors in the serum of SM patients (red) or healthy subjects (blue) quantified and analyzed by Luminex (Millipore). Data shown as mean +/- SEM for n=12 healthy subjects and n=7 SM patients; * p<0.05

- Multiple cytokines, including ILs - 2, 5, 6, 8, 9, 13, 15, 16 and TNFα were significantly elevated in serum of SM patients compared to healthy subjects
- In addition, the chemokines, MIP-1a/b, MCP-3, BCA-1 and the growth factor, TGFα were significantly increased in serum of SM patients

CONCLUSIONS/DISCUSSION

- Siglec-8 was robustly expressed on diseased mast cells and mature eosinophils in bone marrow aspirates from SM patients
- AK002 depleted mature bone marrow eosinophils, which may be valuable to SM patients with associated eosinophilia
- AK002 reduced the expression of SM-associated mast cell surface markers and decreased mast cell mediators in SM patient bone marrow, suggestive of broad mast cell inhibition
- AK002 reduced many cytokines and chemokines in bone marrow that were also elevated in serum of SM patients compared to healthy subjects
- These results suggest that AK002 could represent a novel approach for the treatment of SM

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