

KTN0158, a Humanized Anti-KIT Monoclonal Antibody, Reduces Airway Eosinophilia in a Feline Model of Allergic Asthma

Elizabeth M. Mandel¹, Rich W. Gedrich¹, Chee-Hoon Chang², Julie Trzil², John Dodam², Leah A. Cohn², Christine Lubeski¹, Yaron Hadari¹, Theresa LaVallee¹, Carol R. Reinero²

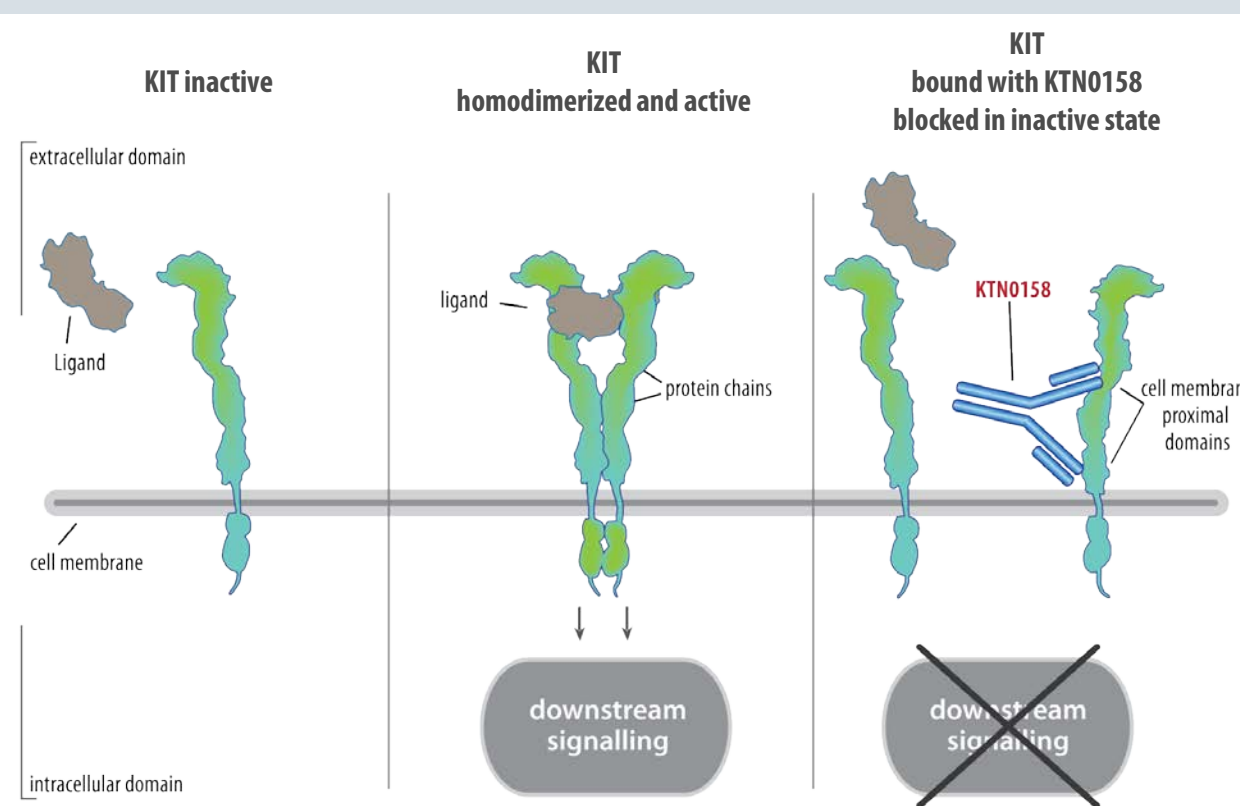
Kolltan Pharmaceuticals, Inc., New Haven, CT¹; College of Veterinary Medicine, University of Missouri, Columbia, MO²



Introduction

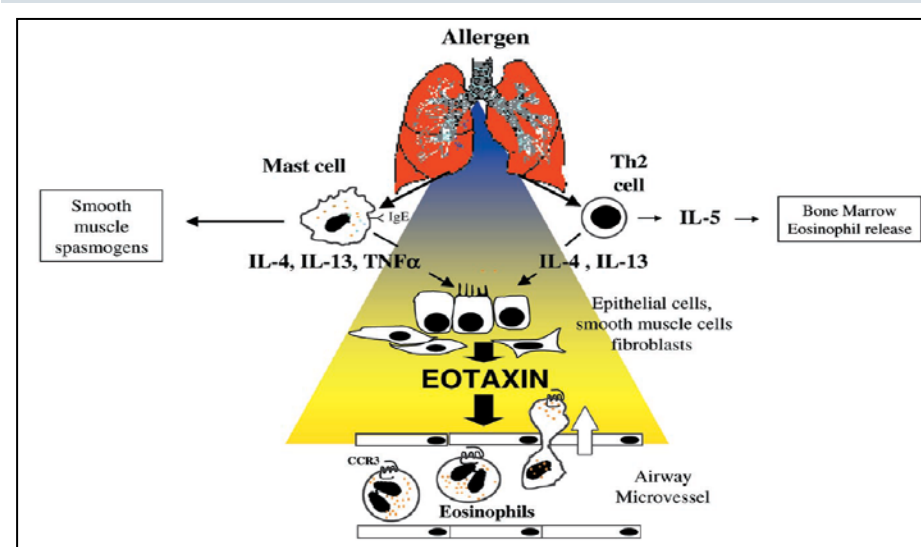
- KTN0158 is a humanized immunoglobulin G1 kappa (IgG1k) monoclonal antibody (mAb) that specifically binds the membrane-proximal D4 domain of KIT (KIT; mast/stem cell factor growth factor; CD117) and inhibits homotypic interactions of the receptor.
- KIT, a receptor tyrosine kinase, and its ligand, stem cell factor (SCF), have multiple effects on mast cells and eosinophils in the lung (Oliveira et al., 2003; Reber et al., 2006).
- Mast cells are predominantly regulated by KIT and the high affinity Fc epsilon receptor 1 binding IgE (Oliveira et al., 2003), and have been implicated in a variety of allergic and inflammatory diseases such as asthma (Metcalf et al., 1997).
- In humans, serum SCF and soluble KIT concentrations correlate with increased asthma severity (Makowska et al., 2009) and the mRNA levels of SCF and KIT are increased in asthmatic patients compared to healthy controls (Al-Muhsen et al., 2004).

Inhibition of KIT Signaling by KTN0158

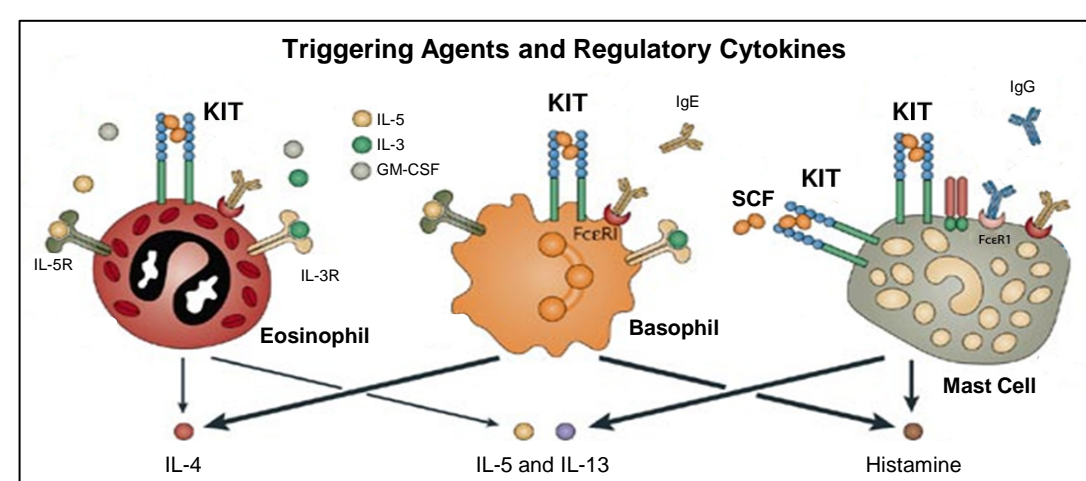


- KTN0158 blocks KIT dimerization and stem cell factor (SCF) ligand binding.

Mast Cells and Eosinophils are Key Mediators of Allergic Asthma/Rhinitis



- Mast cells play a significant role in early and late phase responses in allergic asthma that contribute to airway eosinophilia.

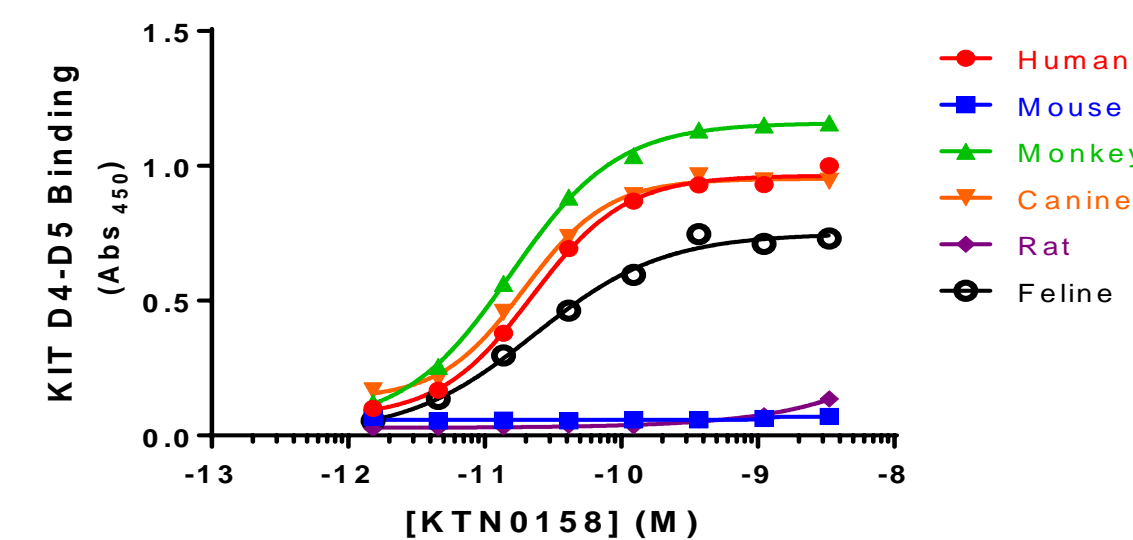


Adapted from: Bischoff S.C. *Nat Rev Immunol* 7:93-104

- KIT is expressed on multiple cell types that are key regulators of the allergic response, including mast cells, eosinophils and basophils.
- Signaling through the KIT receptor contributes to activation of these cell types.
- Mediators released from activated mast cells, eosinophils and basophils cause aggravation of allergic symptoms.

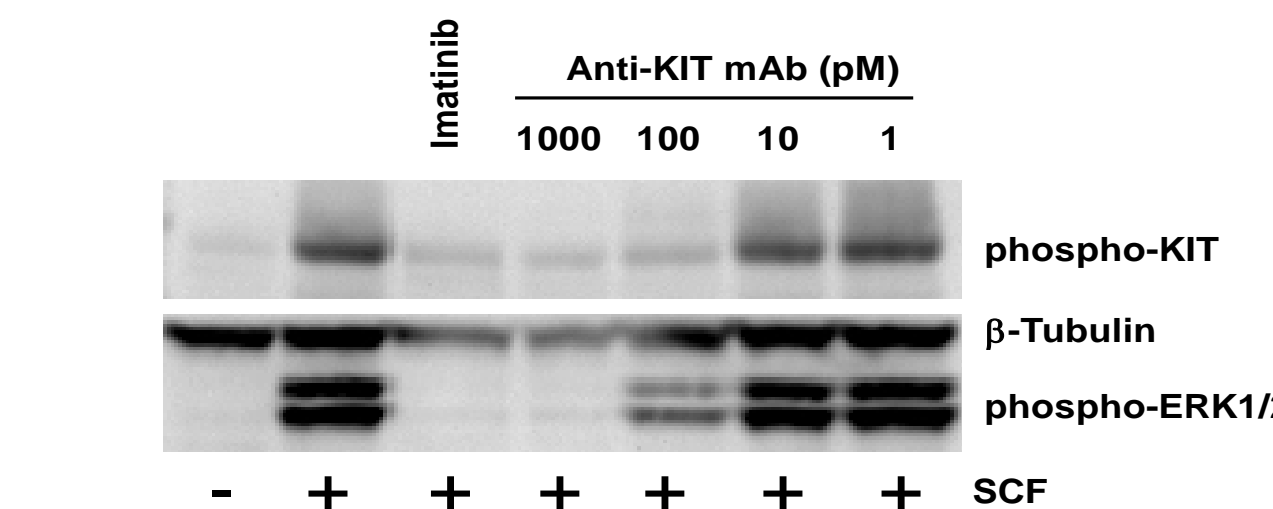
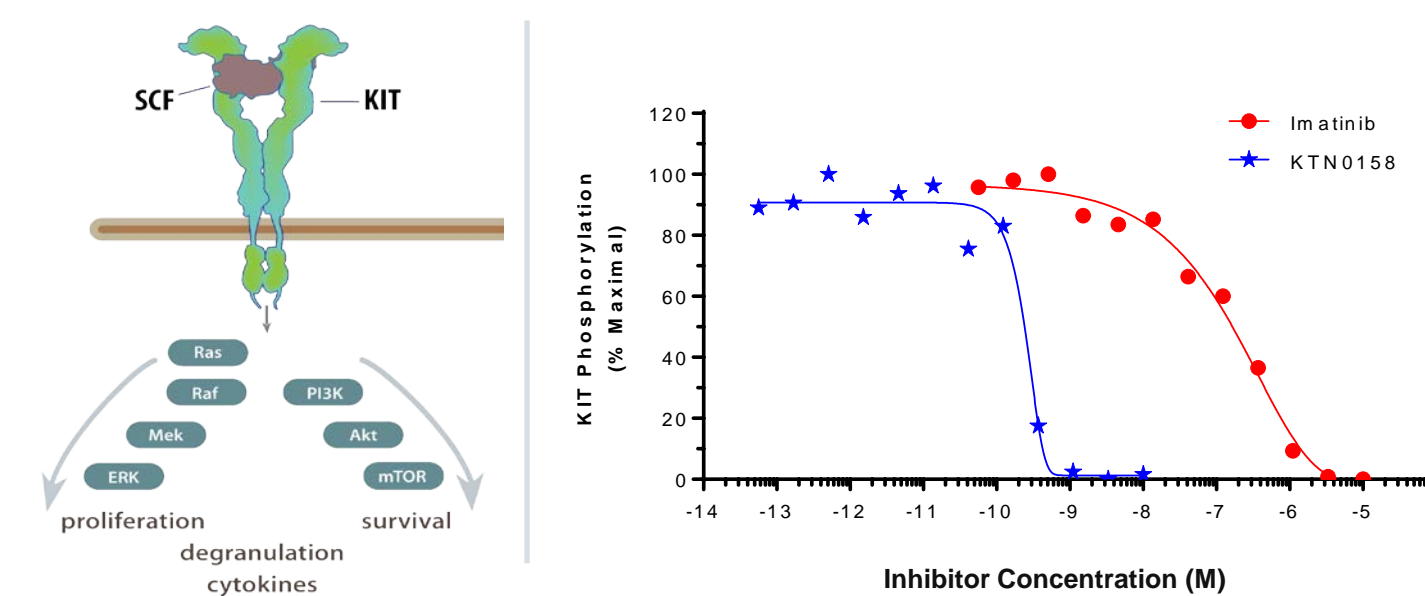
Results

KTN0158 Binds KIT D4-D5 Domains from Various Species



- KTN0158 binds KIT D4-D5 antigens from human, monkey, dog and cat, but does not bind mouse or rat KIT.
- The cat is the only animal species to spontaneously develop allergic asthma with the same hallmark features as human disease, making an experimental feline asthma model using Bermuda grass allergen an excellent preclinical model.

KTN0158 Inhibits SCF-Induced KIT Phosphorylation and Signaling in CHO Cells Expressing Wild-type KIT and in Human H526 Lung Cancer Cells

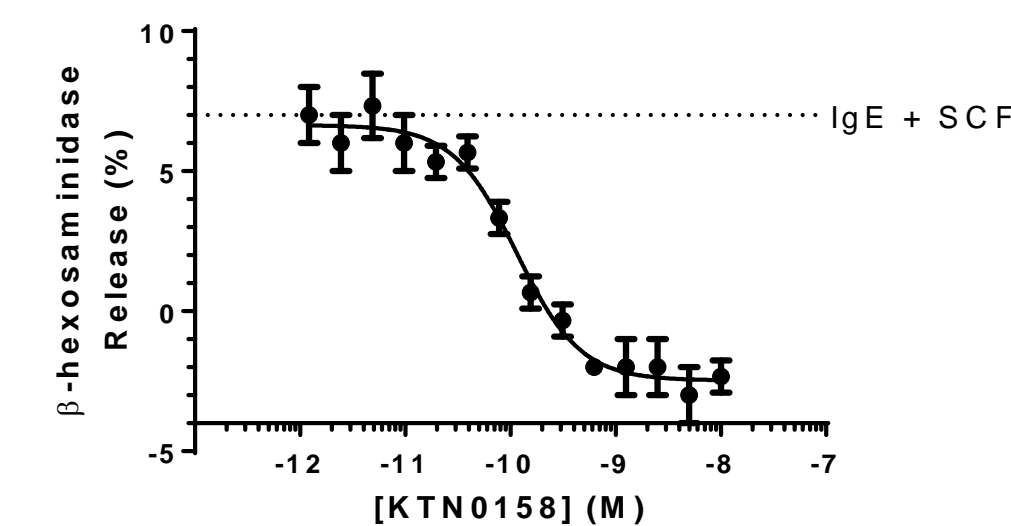


- Stimulation of the KIT receptor by SCF leads to receptor homodimerization and auto-phosphorylation, as well as the induction of downstream signaling pathways responsible for proliferation, survival, degranulation and cytokine release.
- KTN0158 is a significantly more potent inhibitor of SCF-induced KIT phosphorylation than the small molecule inhibitor, Imatinib (IC₅₀=0.13 nM for KTN0158 vs. IC₅₀=263 nM for Imatinib).
- KTN0158 inhibits SCF-induced KIT phosphorylation and subsequent downstream signaling through the MAPK pathway.

References

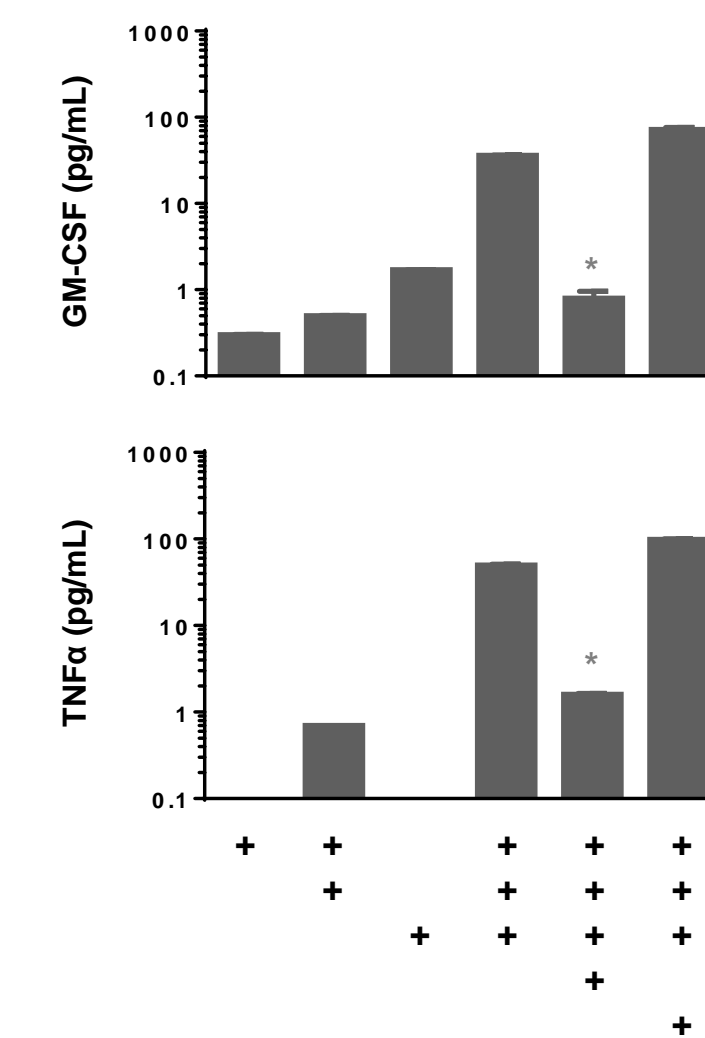
• Metcalfe DD, Baram D, Mekori YA. (1997). *Physiol Rev*. 77:1033-79.
 • Oliveira SH, Lukacs NW. (2003). *Curr Drug targets Inflamm Allergy*. 2:313-318.
 • Reber L, Da Silva CA, Frossard N. (2006). *Eur J Pharmacol*. 533:327-340.
 • Metcalfe DD, Baram D, Mekori YA. (1997). *Mast Cells. Physiol Rev*. 77:1033-79.
 • Makowska JS, Cieslak M, Kowalski ML. (2009). *BMC Pulm Med*. 9:27.
 • Al-Muhsen S, Shablovsky G, Olivenstein R, Mazer B, Hamid Q. (2004). *Clin Exp Allergy*. 34:911-916.
 • Conroy DM and Williams TJ. (2001). *Respir Res*. 2:150-156.
 • Bischoff SC. (2007). *Nat Rev Immunol*. 7:93-104.
 • Norris Reinero C, Decile K, Berghaus R, Williams K, Leutenegger C, Walby W, Schelegle E, Hyde D, Gershwin L. (2004). *Int Arch Allergy Immunol*. 71:583-591.

Degranulation is Inhibited by KTN0158 in the Human LAD2 Mast Cell Line



- KTN0158 is a potent inhibitor of SCF-mediated β-hexosaminidase release caused by mast cell degranulation (IC₅₀=114 pM).

KTN0158 Inhibits SCF-Induced Secretion of TNFα and GM-CSF in the Human LAD2 Mast Cell Line



- Treatment of LAD2 mast cell with KTN0158 inhibits release of TNFα and GM-CSF cytokines following IgE and SCF stimulation.

Methods

Analysis of KIT Phosphorylation by ELISA

Chinese Hamster Ovary (CHO) cells expressing wild-type human KIT were starved, pre-treated for two hours with KTN0158, precursor antibodies to KTN0158 or Imatinib, and stimulated for 10 minutes with SCF ligand. KIT phosphorylation was measured by ELISA using a capture antibody to total KIT and an anti-phospho-tyrosine capture antibody.

Analysis of KIT and ERK Phosphorylation by Western Blot

H526 cells were starved overnight, pre-treated with KTN0158 or Imatinib (1 μM) at the indicated concentrations and then stimulated with SCF for 10 minutes. Lysates (30 mg) were separated by SDS-PAGE and analyzed by Western blot with antibodies recognizing phosphorylated KIT, phosphorylated ERK1/2 and tubulin.

Analysis of Degranulation and Cytokine Production in LAD2 Cells

LAD2 cells were incubated with biotinylated human myeloma IgG1 followed by addition of SCF and then streptavidin to crosslink IgE. Percent β-hexosaminidase release was determined by measurement of β-hexosaminidase in the media compared to the total β-hexosaminidase in the media and lysed cells. TNFα and GM-CSF release was measured in supernatants using multiplexed capture immunoassays with detection by ECL (Meso Scale Discovery, Gaithersburg, MD).

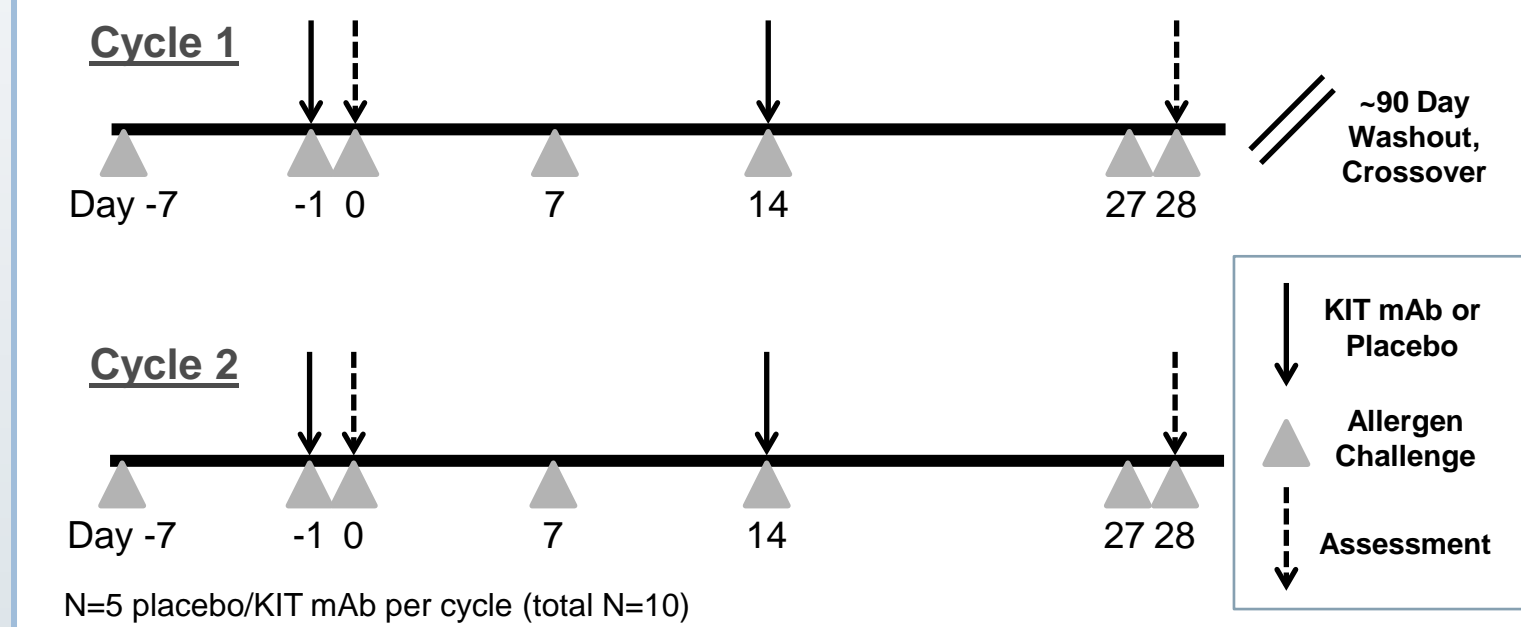
Species Cross-Reactivity

The D4-D5 domains of KIT from human, monkey, cat, dog, mouse and rat were expressed in S19 cells and purified. Equal amounts of protein were coated onto 96-well plates. Binding was measured by direct ELISA using KTN0158 as the detection antibody followed by an anti-human IgG-HRP secondary antibody.

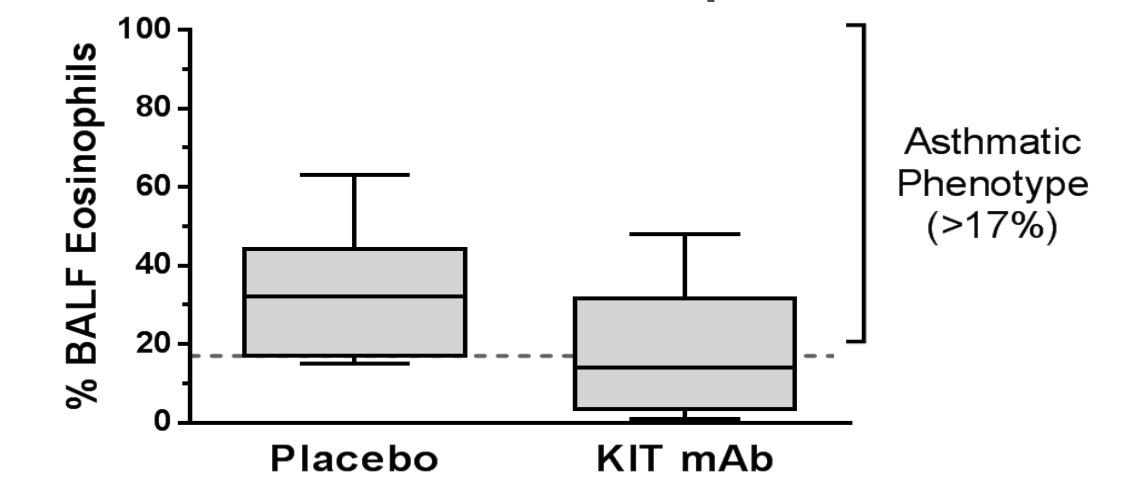
Assessment of KTN0158 in a Feline Model of Allergic Asthma

After induction of experimental asthma and documentation of an asthmatic phenotype (Norris Reinero et al., 2004), 10 experimentally asthmatic cats received bimonthly allergen challenges to induce chronic asthma. Six months later, cats were enrolled in study and randomly assigned to receive either a humanized monoclonal anti-KIT antibody (KTN0158) or placebo (PBS) infusion. The treatment trial began with an aerosol challenge of bermuda grass allergen (BGA) at day -7. On day -1, cats received KTN0158 or placebo (N=5 per group). On day 0, BGA bronchoprovocation in tandem with ventilator-acquired pulmonary mechanics followed immediately by BALF collection was performed to study the early asthmatic response (EAR) to allergen. Administration of additional aerosol challenges of BGA were performed on days 7, 14 and 27. Infusion of KTN0158 or placebo was repeated on day 14. Ventilator acquired pulmonary mechanics using methacholine as the bronchoprovocant followed immediately by collection of BALF were performed on day 28 to assess the late phase reaction one day post-BGA challenge. There was a 2 month washout before crossover to the alternate treatment (placebo or KTN0158) with identical study design.

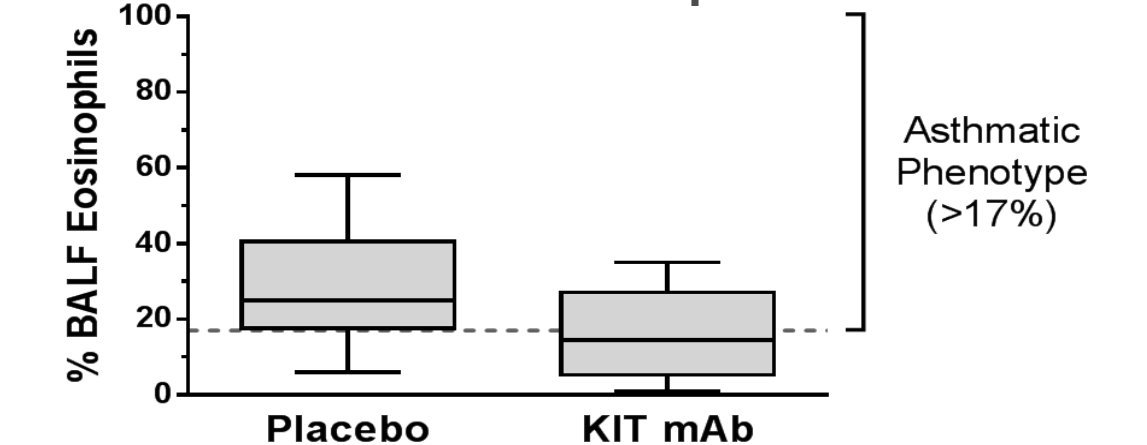
KTN0158 Reduces Airway Eosinophilia in a Feline Model of Allergic Asthma



Acute Asthma Response



Chronic Asthma Response



- Bermuda grass allergen (BGA) challenge and subsequent immediate collection of bronchoalveolar lavage fluid (BALF) demonstrated significant (p<0.05) reduction of airway eosinophilia during the early asthmatic reaction (acute) in KTN0158-treated cats compared with placebo.
- Significant (p<0.05) reduction of airway eosinophilia was also noted in the late-phase asthmatic response (chronic) on day 28, 24 hours after BGA challenge in KTN0158-treated cats compared with placebo.

Conclusions

- KTN0158 is a potent inhibitor of SCF-induced KIT signaling.
- KTN0158 modulates SCF-mediated effects on degranulation and cytokine release in the LAD2 mast cell line.
- KTN0158 treatment decreased airway eosinophil numbers in both acute and chronic phases of a feline allergic asthma model.
- Collectively, these data suggest that KTN0158 can modulate mast cell and eosinophil function via KIT and warrant further evaluation of KTN0158 as a treatment in mast cell-related diseases such as asthma.
- Studies to investigate the potential benefit of KTN0158 in mast cell-related diseases and inflammation, and to evaluate safety are planned.

