

Regulation of Mast Cell Activity by KTN0158, a Humanized anti-KIT Monoclonal Antibody

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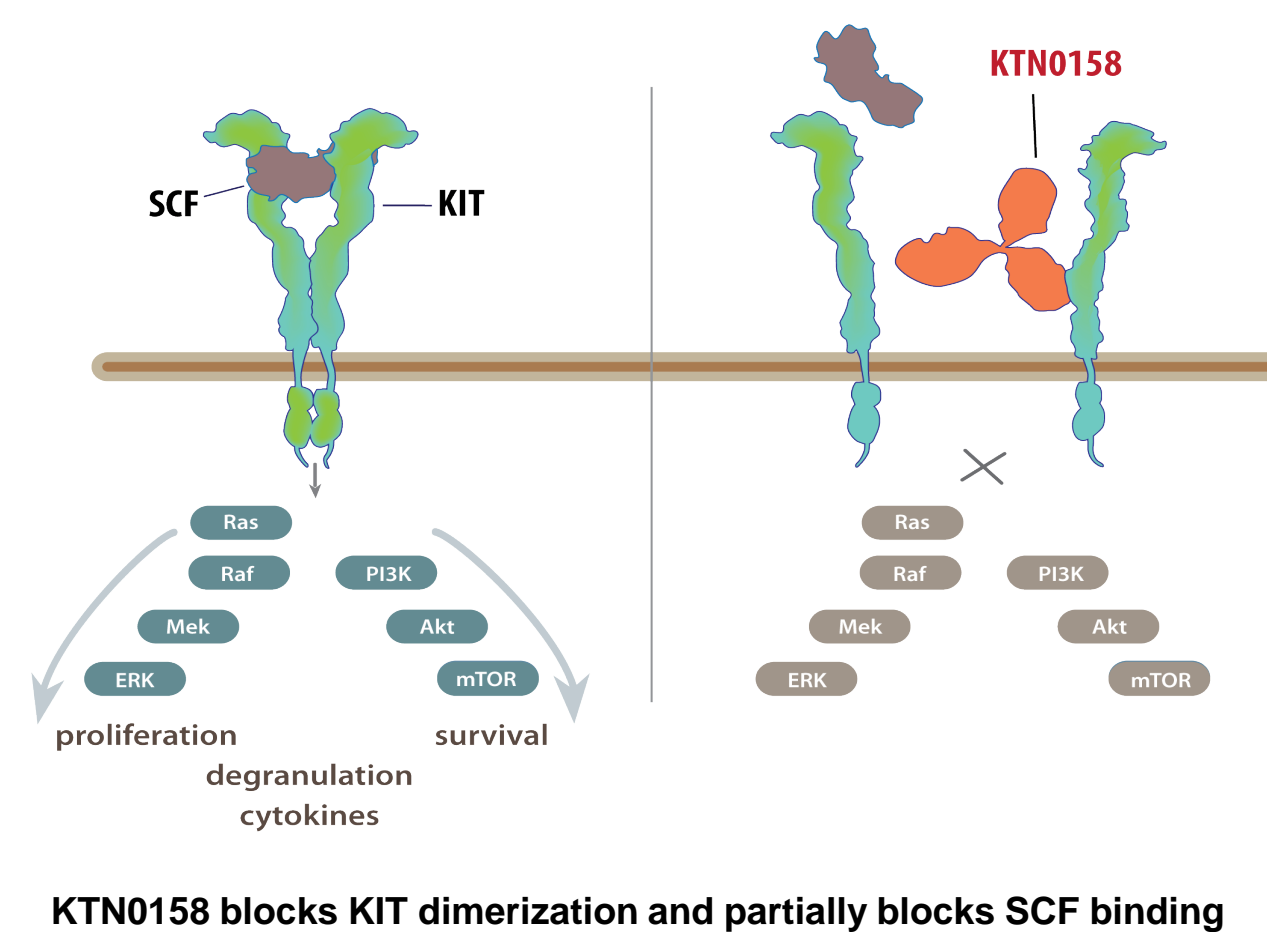
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Introduction

- KTN0158 is a humanized immunoglobulin G1 kappa (IgG1κ) monoclonal antibody (mAb) that specifically binds KIT (c-KIT; mast/stem cell factor growth factor; CD117).
- Mast cells have been implicated in a variety of allergic and inflammatory diseases such as asthma and rheumatoid arthritis as well as fibrosis (Metcalf et al., 1997).
- Mast cell infiltrates are associated with tumors and may have roles in tumor promotion or rejection depending on the setting (Theoharides and Conti, 2004).
- KIT signaling in mast cells appears to play an important role in the development of plexiform neurofibromas in neurofibromatosis type 1 (NF1; Staser et al., 2012).

Inhibition of KIT Signaling by KTN0158



Effects of Imatinib on a Highly Morbid Neurofibroma in a Pediatric Patient

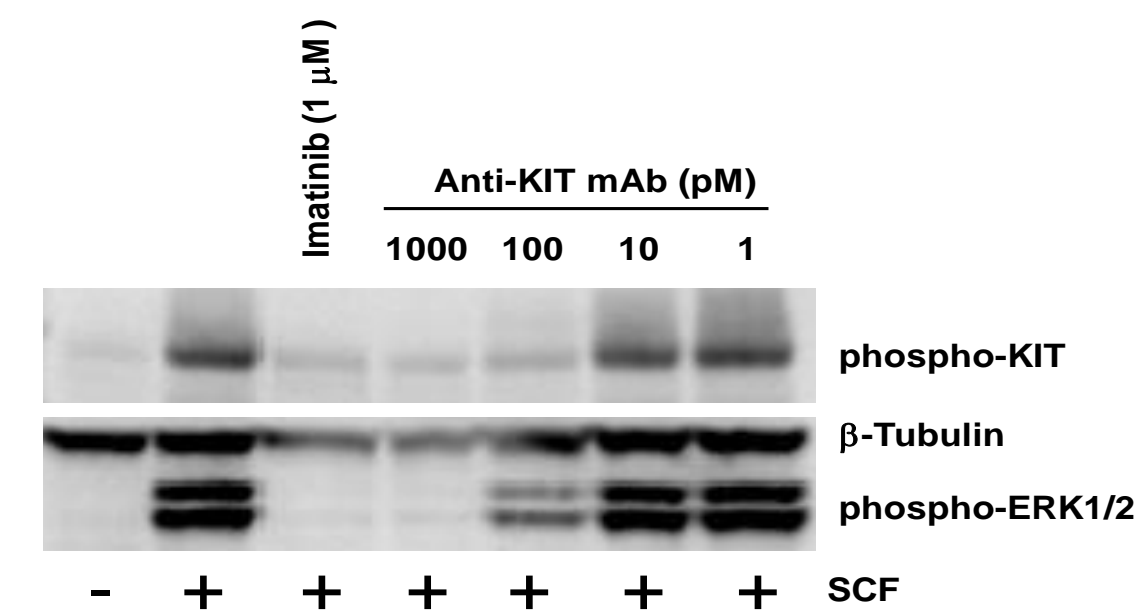


Yang et al., Cell 135:437-448.

Inhibition of KIT may provide benefit in treatment of plexiform neurofibromas

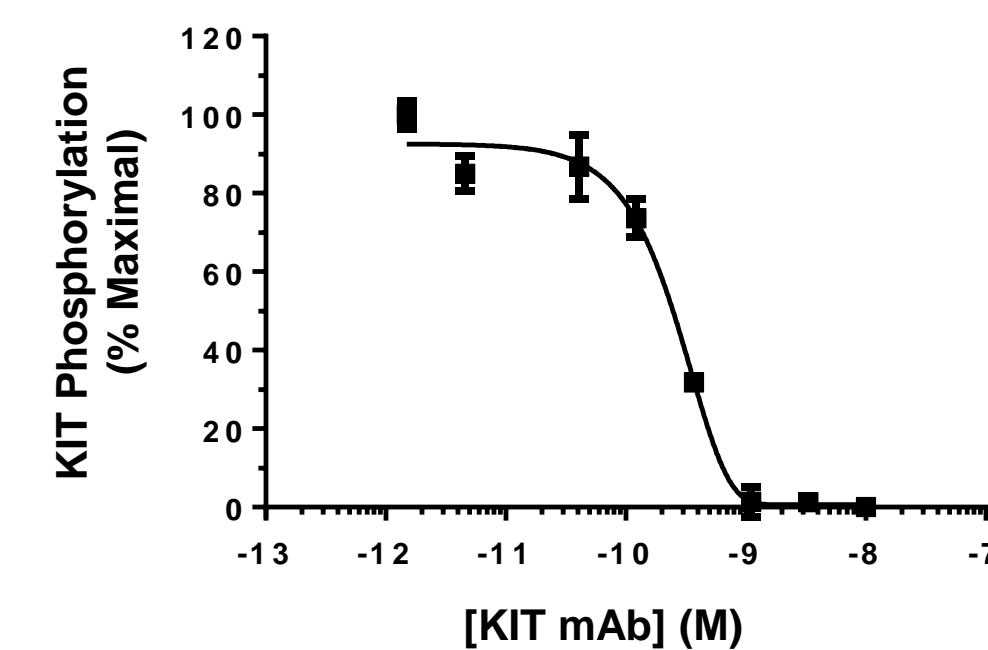
Results

Effects of KTN0158 on SCF-Induced KIT Activation and Signaling in H526 Small Cell Lung Cancer Cells



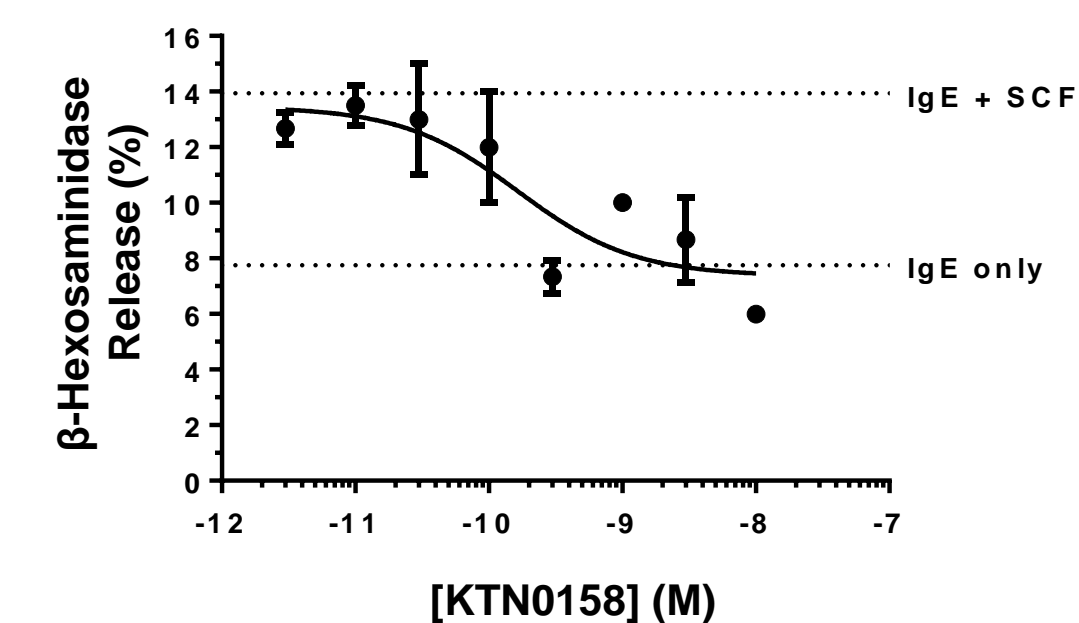
KTN0158 is a potent inhibitor of SCF-induced KIT phosphorylation and downstream signaling via the MAPK pathway

Effects of KTN0158 on SCF-Induced KIT Phosphorylation in CHO Cells Expressing Wild-type KIT



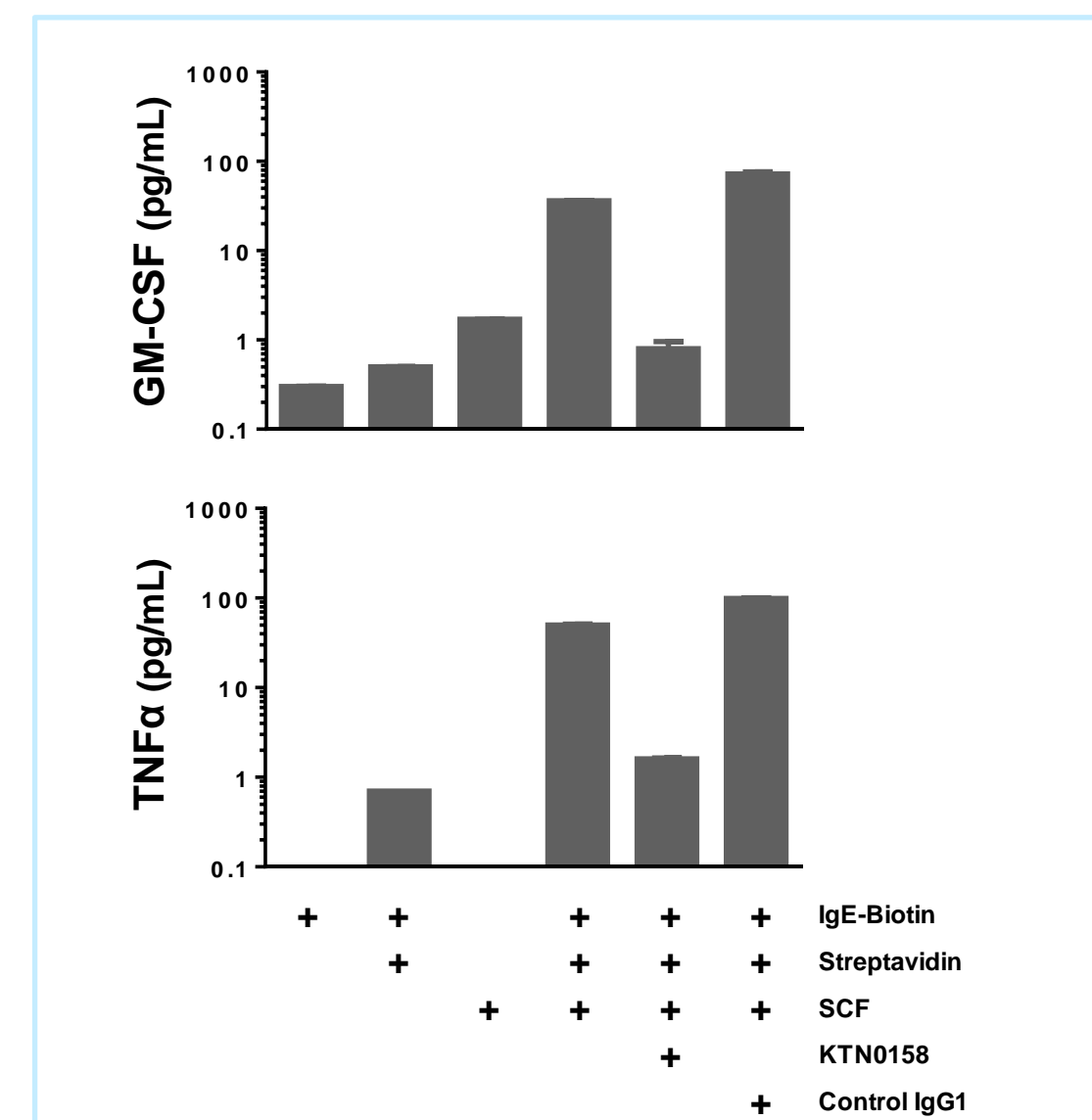
- KTN0158 is a potent inhibitor of SCF-induced KIT phosphorylation
- IC₅₀ = 169 pM (n = 13)

Effects of KTN0158 on Degranulation in the Mast Cell Line LAD2



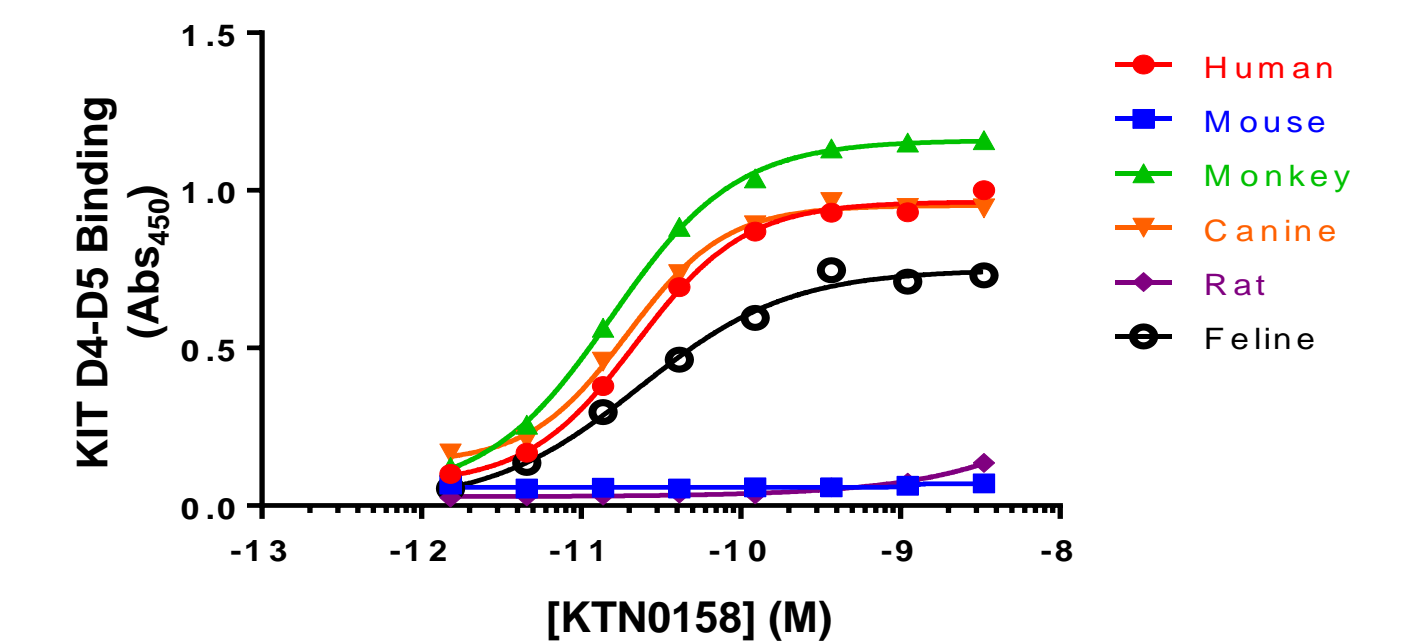
KTN0158 is a potent inhibitor of SCF-mediated effects on degranulation

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



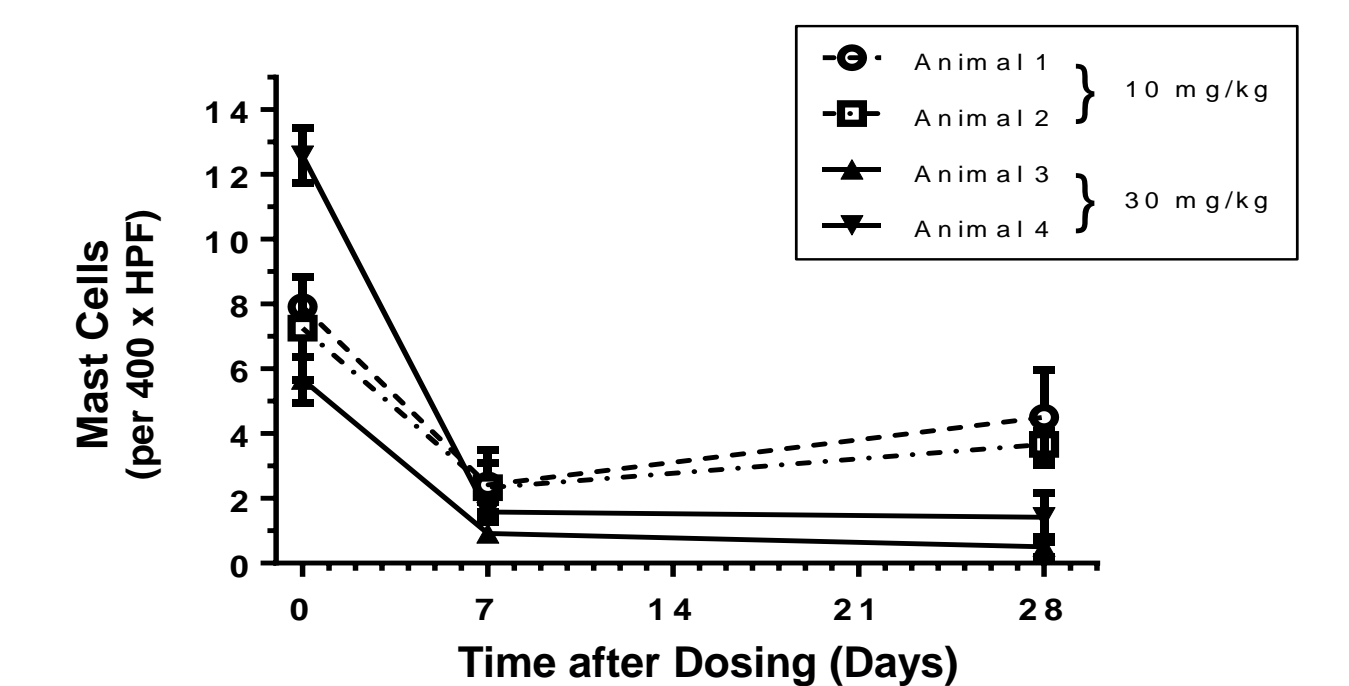
KTN0158 is a potent inhibitor of SCF-mediated effects on cytokine secretion

Cross-reactivity with KIT from Various Species



- Binding was observed with human, monkey, dog and cat KIT
- No binding was observed with mouse or rat KIT

Effect of KTN0158 on Mast Cell Numbers in Dog Skin



- Substantial decreases in mast cells were observed 1 week after dosing in both groups
- Evidence of recovery was observed 4 weeks after dosing in the 10 mg/kg group, suggesting a dose-related effect

Methods

Analysis of KIT and ERK Phosphorylation by Western Blot
H526 cells were starved overnight, pre-treated with KTN0158 or imatinib 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 KIT Phosphorylation by ELISA
Chinese Hamster Ovary (CHO) cells expressing wild-type human KIT were starved, pre-treated for two hours with KTN0158 or precursor antibodies to KTN0158 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 Degranulation and Cytokine Production in LAD2 Cells
LAD2 cells were incubated with biotinylated human myeloma IgE overnight. Cells were pretreated with KTN0158 or control 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 Sf9 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.

Evaluation of KTN0158 in Healthy Research Dogs
Study Design:
A non-GLP pilot study was conducted in healthy research dogs. A total of 4 healthy, 1-year old, unrelated hound dogs weighing between 20-25 kg were assigned to 2 dose groups (1 male, 1 female per group) that were administered a single dose of either 10 or 30 mg/kg KTN0158 intravenously. The dogs were followed for 28 days.

Mast Cells Assessment in Skin:
Four 8 mm punch biopsies were collected from skin on the dorsum prior to dosing and on Days 7 and 28. Biopsies were fixed in formalin, embedded in paraffin and stained with hematoxylin and eosin and Toluidine blue. Mast cells with metachromatic granules were counted in three random 400x fields in each biopsy sample (n = 4), including superficial dermal, periaxonal, and deep dermal areas. Data are reported as means of the 4 biopsies ± SEM.

References

- Metcalfe DD, Baram D, Mekori YA. (1997). Mast Cells. *Physiol Rev.* 77:1033-79.
- Staser K, Yang FC, Clapp DW. (2012). Pathogenesis of plexiform neurofibroma: tumor-stromal/hematopoietic interactions in tumor progression. *Annu Rev Pathol.* 7:469-95.
- Theoharides TC, Conti P. (2004). Mast cells: the Jekyll and Hyde of tumor growth. *Trends Immunol.* 25:235-41.

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Conclusions

- KTN0158 is a potent inhibitor of SCF-induced KIT signaling.
- KTN0158 modulates SCF-mediated effects on degranulation and cytokine production in the mast cell line LAD2.
- KTN0158 treatment decreased mast cell numbers in dog skin indicating that sufficient concentrations of KTN0158 were achieved in skin to inhibit KIT signaling in mast cells.
- Collectively, these data suggest that KTN0158 can modulate mast cell function via KIT and may provide therapeutic benefit in mast cell-related diseases such as NF1.
- Studies to investigate the potential benefit of KTN0158 in mast cell-related diseases such as NF1 and to evaluate safety are planned.

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