KTN0158, a Humanized Anti-KIT Monoclonal Antibody, Demonstrates Antitumor Activity in Dogs with Mast Cell Tumors

The Ohio State University College of Veterinary Medicine

Cheryl London[1], Sarah Rippy[1], Heather Gardner[1], Gerald Post[2], Neal Janson[3], Linda Crew[3], Theresa LaVallee[3], Richard Gedrich[3]

The Ohio State University, Columbus, OH, USA[1]; The Veterinary Cancer Center, Norwalk CT, USA[2]; Kolltan Pharmaceuticals, Inc., New Haven, CT, USA[3]

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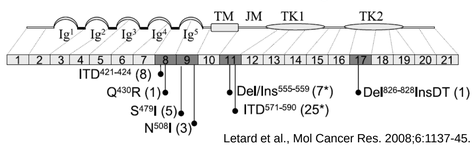
Introduction

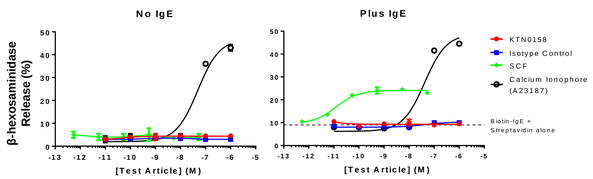
- KTN0158 is a humanized anti-KIT IgG1 monoclonal antibody that is being developed as a potential therapy for cancer and mast cell-related diseases such as neurofibromatosis type 1 (NF1)
- KTN0158 properties:
  - Binds canine, feline, non-human primate and human KIT with high affinity
  - Potent inhibitor of wild-type and some oncogenic variants of KIT
  - Modulates mast cell function and survival *in vivo* in dogs, cynomolgus monkeys and cats
- A clinical trial was conducted in dogs with spontaneous mast cell tumors (MCT) where KIT is known to drive tumor cell survival and growth, in part via activating mutations similar to those found in human gastrointestinal stromal tumors (GISTs)

Mutations Detected in Dog MCTs



Letard et al., Mol Cancer Res. 2008;6:1137-45.

KTN0158 Does Not Have Agonist Activity *In Vitro*



- No degranulation in primary human mast cells *in vitro* at KTN0158 concentrations up to 1000 nM with or without IgE crosslinking
- KTN0158 did not induce KIT phosphorylation in cells expressing endogenous or exogenous KIT

Methods

***In Vitro* Assays**

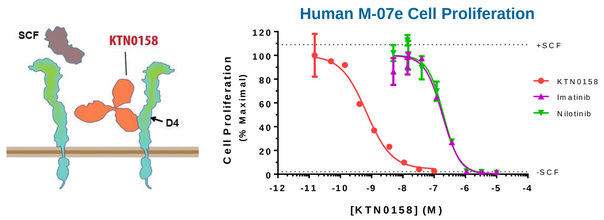
- Human mast cells: primary human mast cells were differentiated *in vitro* from peripheral blood mononuclear cells by standard methods (Saito et al, Nat Protoc. 1:2178-83). Mast cells were treated with serial dilutions of the test articles indicated and degranulation was measured by β-hexosaminidase release.
- Proliferation assays: The human acute megakaryoblastic leukemia cell line M-07e (obtained from DSMZ GmbH; Avanzi et al, Br J Haematol. 69:359-66) was grown in the presence of SCF and cells were treated with serial dilutions of KTN0158, imatinib or nilotinib. Proliferation was measured using the CellTiter-Glo® assay (Promega).
- Dog KIT Phosphorylation: HEK 293 cells were transiently transfected with an expression vector containing the full length dog KIT cDNA. Transfected cells were incubated with canine SCF, KTN0158 and imatinib as indicated. KIT phosphorylation was measured by ELISA.

Clinical Study

- Thirteen dogs with measurable MCT were enrolled into this open-label clinical trial. Twelve dogs were evaluable for efficacy.
- Three dose levels and 2 schedules were evaluated. KTN0158 was administered intravenously.
  - One Dose Schedule: 10 or 30 mg/kg given on Day 0 only
  - Two Dose Schedule: 1 or 10 mg/kg given on Days 0 and 21
- Serial tumor biopsies and blood samples for PK and PD analysis were collected pre- and post-treatment throughout the study.
- Weekly assessments included physical examination and standard laboratory tests (serum chemistries, hematology profiles, and urinalyses) for clinical toxicities and response. Antitumor efficacy was based on objective tumor assessments made according to established RECIST criteria for solid tumors in dogs.
- Adverse events (AEs) were recorded and graded according to VCOG-CTCAE v.1.1 criteria for AEs in dogs.

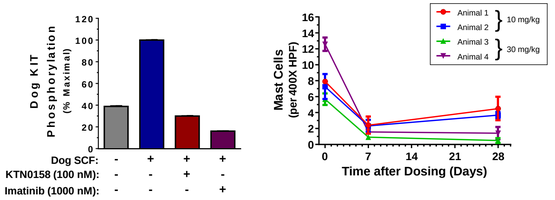
Results

KTN0158 Mechanism of Action and Activity



- KTN0158 binds the Ig-like Domain 4 (D4) of the KIT extracellular domain and blocks KIT homodimerization and ligand binding
- KTN0158 is a more potent inhibitor of SCF-dependent M-07e cell proliferation than imatinib or nilotinib

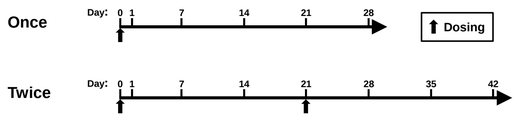
KTN0158 inhibits Dog KIT Activation *In Vitro* and Decreases Mast Cell Numbers in Dog Skin *In Vivo*



- Dose-dependent effect on mast cells *in vivo* (partial recovery at low dose)

Dog MCT Study Design

Schedules

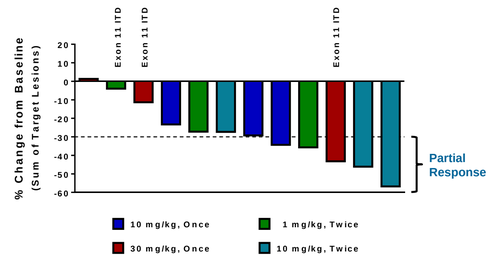


Dosing Cohort	No. of Dogs	Dose (mg/kg)	Schedule
1	3	10	Once (Day 0)
2	3	30	Once (Day 0)
3	3	10	Twice (Day 0 & 21)
4	4*	1	Twice (Day 0 & 21)

* One dog not evaluable for efficacy

- Outcome measures included preliminary assessment of efficacy, safety, PK, KIT mutation status and PD biomarkers

Best Tumor Response in Dogs with Evaluable MCTs



- All dogs treated with KTN0158 experienced clinical benefit

Lack of Neoplastic Mast Cells in a Subset of Primary Tumors and Metastatic Lymph Nodes after KTN0158 Treatment

Dose Group	Patient No.	KIT Mutation	Best Response (% Change)	Primary Tumor Post-treatment Histopathology	Lymph Node Pretreatment Cytology	Lymph Node Post-treatment Histopathology
10 mg/kg (Day 0)	1	Negative	-23.3	MCT	Not Done	Not Done
	2	Negative	-29.2	**NED**	Metastatic	**NED**
	3	Negative	-34.3	MCT	Metastatic	**NED**
30 mg/kg (Day 0)	4	Exon 11	-11.3	**NED**	Not Done	Not Done
	5	Negative	1.3	MCT	Not metastatic	Not Done
	7	Exon 11	-43.2	Not Done	Metastatic	Not Done
10 mg/kg (Day 0 & 21)	6	Negative	-46.1	MCT	Not Done	MCT
	8	Negative	-56.8	MCT	Not Done	Not Done
	9	Negative	-27.3	MCT	Not metastatic	Not Done
1 mg/kg (Day 0 & 21)	11	Negative	-27.2	MCT	Not Done	Not Done
	12	Negative	-35.7	MCT	Metastatic	**NED**
	13	Exon 11	-4.0	MCT	Metastatic	MCT

NED = no evidence of disease; MCT = mast cell tumor

Adverse Events in Dogs with MCTs after KTN0158 Treatment

Hematological Adverse Events

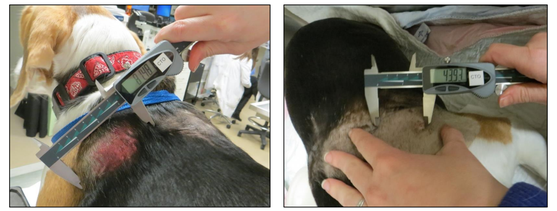
Dose Group	Toxicities (number of events by grade)											
	Anemia				Thrombocytopenia				Neutropenia			
	1	2	3	4	1	2	3	4	1	2	3	4
10 mg/kg once	1	1				1	1		2		1	
30 mg/kg once	2	1			1	1	2				1	2
10 mg/kg twice	2					2			1	1		
1 mg/kg twice	1					1						

Other Adverse Events

Dose Group	Toxicities (number of events by grade)																			
	ALP Elevation				AST Elevation				ALT Elevation				Hypersensitivity reaction				Edema / erythema			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
10 mg/kg once							1		1	1										
30 mg/kg once	2	2							2								1	1	1	
10 mg/kg twice	2	1					1	1	2		1					1				
1 mg/kg twice	1									1				1						

- Dose-limiting toxicities observed at 30 mg/kg in dogs; however, no significant toxicological findings were observed in multiple studies in cynomolgus monkeys after repeat dosing of KTN0158 with a NOAEL of 75 mg/kg (exposure similar to 30 mg/kg in dogs)
- Maximum tolerated dose (MTD) = 10 mg/kg
- Two dogs experienced anaphylaxis during the second infusion which may have been due to the development of anti-KTN0158 antibodies
- All study related adverse events were transient and recovered fully by study completion

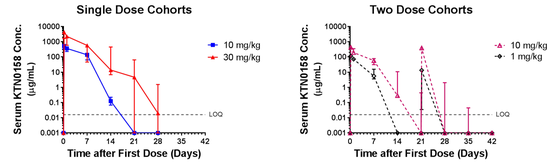
Partial Response to KTN0158 Treatment



Day 1 Day 15

Change in Tumor: - 46.1%

Pharmacokinetics:
Median KTN0158 Serum Concentrations and Anti-Drug Antibodies



- Dose-related increases in exposure were observed after the first and second doses of KTN0158
- Anti-KTN0158 antibodies were detected in 10 of 12 dogs by Day 14 and all dogs at study completion

Conclusions

- **Clinical benefit of KTN0158 was observed in dogs with MCTs at all dosing levels and in tumors with and without activating KIT mutations**
  - 5 dogs with partial responses and 7 dogs with stable disease
  - Histopathology after study completion showed a lack of neoplastic cells in primary tumors and/or lymph node samples from a total of 4 dogs
- **Reversible hematologic and biochemical effects were observed in dogs receiving 10 and 30 mg/kg/dose of KTN0158 with the MTD established at 10 mg/kg**
- **In contrast to findings in dogs, IND enabling toxicology studies in cynomolgus monkeys revealed no significant findings after repeat dosing of KTN0158 with a no observed adverse event level (NOAEL) of 75 mg/kg, the highest dose tested**
- **Overall, nonclinical toxicology demonstrates a favorable safety profile supporting planned clinical testing in human phase 1 studies in 2016 in GISTs and other solid tumors**

Acknowledgements

We would like to acknowledge The Ohio State University Center for Clinical and Translational Science and CTSA Grant number (UL1TR001070) and NIH CCC Grant P30 CA016058.