Efficacy of THB001, a Potent and Selective Oral Small Molecule Inhibitor of Wild Type KIT Receptor Tyrosine Kinase, in a Rat Passive Cutaneous Anaphylaxis Model

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ABSTRACT

Rationale: Mast cells play a central role in the pathophysiology of allergic inflammation. Mast cell activation, migration, proliferation and survival are dependent on KIT (CD117) signaling. THB001 is a potent and selective inhibitor of wild type KIT that has been studied clinically[1]. The current study correlated target engagement, mast cell depletion and efficacy in an in vivo model of anaphylaxis.

Methods: Potency and selectivity were assessed in kinase-dependent Ba/F3 cell proliferation assays. Plasma pharmacokinetics, the pharmacodynamic effect on mast cells in the ear, and inhibition of Evans blue dye extravasation in response to allergen sensitization and challenge were assessed following 28 days of once daily oral administration of 2.5, 10 and 50 mg/kg/day THB001 in a rat model of passive cutaneous anaphylaxis (PCA).

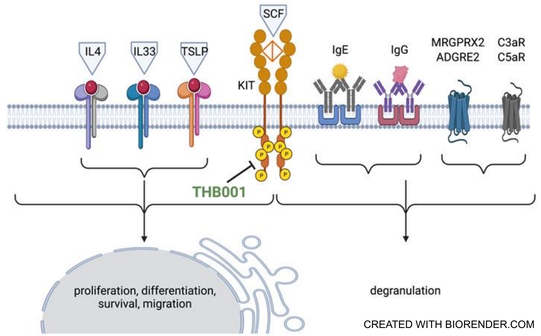
Results: THB001 potently inhibited KIT in cells (IC_{50}=0.02 μM) and had 48- and >100-fold selectivity against CSF-1R and PDGFRα/β, respectively. In the PCA model, THB001 trough plasma concentrations exceeded the protein binding adjusted Ba/F3 IC_{50} for KIT by approximately 2x, 5x and 20x at 2.5, 10 and 50 mg/kg/day, respectively. THB001 resulted in a dose-dependent depletion of dermal mast cells correlating to efficacy at all doses tested, achieving 93% inhibition at 50 mg/kg/day (p<0.0001). Efficacy at the highest THB001 dose was similar to the antihistamine positive control desloratadine and approached the level of response in non-sensitized animals.

Conclusions: THB001, a potent and selective inhibitor of wild type KIT, demonstrated efficacy in a rat PCA model at clinically relevant exposures.

BACKGROUND

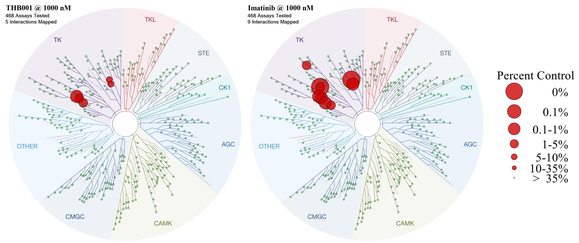
- Mast cells play an important role in the pathophysiology of allergic and chronic inflammatory disease
- Mast cells are critically dependent on KIT signaling in response to stem cell factor (SCF) for their survival, activation and proliferation
- Mast cell activation (including granule release) through Immunoglobulin E (IgE), complement, or cytokines is enhanced by SCF signaling via KIT
- Disruption of KIT signaling through genetic ablation, or steric interference of SCF binding, results in mast cell depletion
- THB001 is a novel and potent small molecule KIT inhibitor with excellent selectivity against other members of the receptor tyrosine kinase family
- The impact of KIT inhibition was evaluated for its ability to deplete tissue mast cells and to prevent anaphylaxis in a rat model of passive cutaneous anaphylaxis

Figure 1. Mast cell activation and function of THB001



RESULTS

Figure 2. THB001 is a highly selective small molecule KIT inhibitor



TREE*spot* kinome interaction S(10) visualization of THB001 and imatinib at 1 μM. Kinome selectivity of THB001 was profiled at 1 μM against 468 kinases using the DiscoverX KINOMEscan platform.[2] Imatinib was profiled at the same concentration for comparison. S(X)= number of non-mutant kinase interactions within X% of positive control ligand.

- THB001 had the lowest possible S(1) selectivity score with KIT being the only non-mutant kinase interaction <1% of control
- The S(10) selectivity level contained CSF-1R, DDR1, DDR2 and PDGRFβ as potential anti-targets of interest for THB001
- Imatinib profiling indicated 6 interactions at the S(1) selectivity level including ABL1, CSF1R, DDR1, DDR2, KIT and PDGFRβ. Additional S(10) interactions mapped included LCK and PDGFRα
- To assess the functional significance of the mapped THB001 S(10) interactions, K_D values were determined with the DiscoverX KINOMEscan platform. CSF-1R and PDGFRβ were selected for further evaluation in cellular assays based on the biochemical fold selectivity
- Cellular assays included cell viability, NanoBRET assay[3] for KIT and CSF-1R, as well as a Phospho-ELISA monitoring of KIT inhibition and PDGFRβ phosphorylation

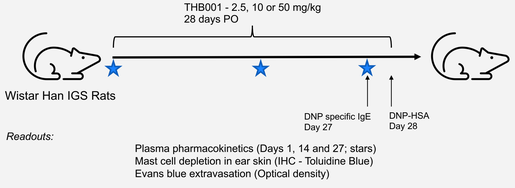
Taken together, these data demonstrate the high selectivity of THB001 for KIT with low engagement of off-target kinases expected at pharmacologically relevant concentrations.

Table 1. THB001 is a highly selective and potent KIT inhibitor

Assay/ Target	KIT	CSF-1R	PDGFRβ	DDR1	DDR2
KINOMEscan K_D (nM)	0.4	34	21	68	60
Cellular viability Parental cell lines[a] EC_{50} (nM)	63	>3,000	>3,000	ND	ND
Cellular viability Engineered cell lines[b] EC_{50} (nM)	20	950	2,100	ND	ND
NanoBRET[c] EC_{50} (nM)	30	1,510	NA	ND	ND
Phospho-ELISA[d] IC_{50} (nM)	2.6	ND	838	ND	ND

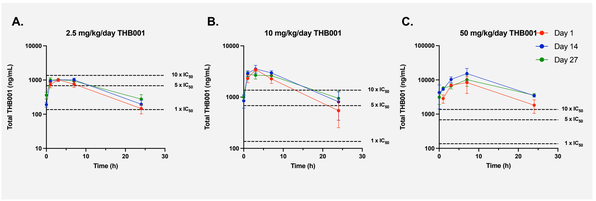
THB001 potency in biochemical and cellular assays. KINOMEscan biochemical assessments were performed using the DiscoverX proprietary active site-dependent competition binding assay. [a]; Cell viability assays were assessed using CellTiter-Glo in KIT dependent M-07e cells, CSF1R dependent M-NFS-60 cells and PDGFRβ dependent A10 cells. [b]; Cell viability as also determined in Ba/F3 murine B cells with ectopically expressed KIT, CSF1R and PDGFRβ. [c]; NanoBRET (HEK293) assay was performed to profile cellular target occupancy (note: PDGFRβ is unavailable in NanoBRET format). [d]; Phospho-ELISA assays to determine the level of target inhibition were performed on extracts of M-07e cells (KIT) and NIH3T3 cells (PDGFRβ) stimulated with THB001. NA: assay not available, ND: not determined.

Figure 3. Pharmacokinetic/Pharmacodynamic activity and efficacy of THB001 in the rat Passive Cutaneous Anaphylaxis model



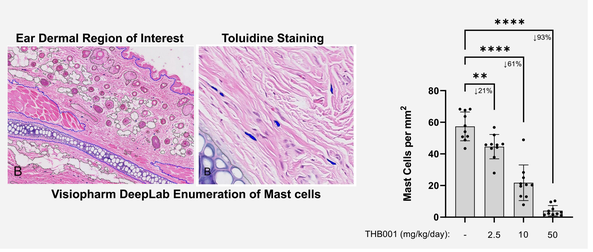
Passive Cutaneous Anaphylaxis (PCA) model design. Wistar Han rats were treated with THB001 (2.5, 10 or 50 mg/kg) daily for 28 days. Cohorts were sensitized with anti-DNP specific antibodies on day 27 and evaluated for anaphylaxis following DNP-HSA (dinitrophenyl hapten-human serum albumin) challenge on day 28. Readouts include mast cell depletion, tissue anaphylaxis (Evans Blue extravasation) and serum PK analysis (performed on days 1, 14 and 27).

Figure 4. Daily oral administration of THB001 results in sufficient systemic exposure for KIT inhibition



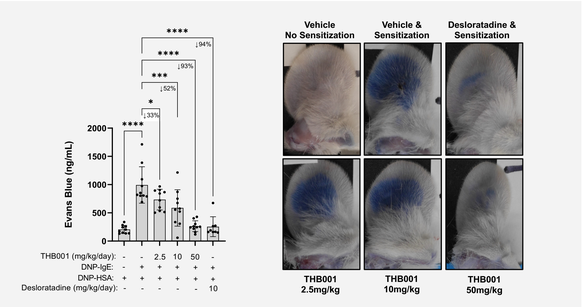
THB001 trough plasma concentrations exceeded cellular KIT IC_{50}. THB001 was administered to rats at 2.5, 10 and 50 mg/kg/day (panels A, B and C respectively). Plasma exposures of THB001 were determined on study days 1, 14 and 27. Multiples of the in vitro KIT IC_{50} (adjusted for plasma protein binding [rat plasma protein binding 94.5%]) are shown. THB001 exposures exceeded the KIT *in vitro* IC_{50} by more than 1-fold at 2.5 mg/kg/day, approximately 5-fold at 10 mg/kg/day and more than 10-fold at 50 mg/kg/day for the entire duration of the study.

Figure 5. Dose-Dependent depletion of mast cells following THB001 treatment



Dose dependent depletion of mast cells following treatment with THB001. Mast cell density was determined in ear tissue (skin) from THB001 treated rats. (Left) Mast cell enumeration was determined using Luna's Toluidine blue (LTB) staining of fixed, bisected ear biopsies with Visiopharm DeepLab neural network algorithm-based enumeration. (Right) Mast cell density for treated animals. Individual animals are represented by black dots; grey bars represent mean for each treatment group and error bars represent standard deviation for each group. Statistics compared THB001 treated groups to the vehicle control group via ordinary one-way ANOVA (F = 69.42, p<0.0001) and Dunnett's multiple comparisons test using GraphPad Prism version 9.4.1. P- values represented by symbols as follows: p<0.01 (**), P<0.0001 (****). Percentage change in mast cell density relative to vehicle treated animals indicated by the number adjacent to the pair-wise comparison lines.

Figure 6. Dose-Dependent Decrease in skin anaphylaxis following THB001 treatment



Dose-Dependent Decrease in skin Anaphylaxis Following THB001 Treatment. Evans blue dye extravasation in the ears of sensitized and THB001 or Desloratadine (control) treated rats. Representative ear images are included (right). Evans blue dye extravasation for individual animals is represented by black dots and grey bars represent the mean for each treatment group (left). Error bars represent standard deviation for each group. Statistics compared THB001 treated groups and the vehicle control group to the antibody sensitized/antigen challenged group via ordinary one-way ANOVA (F = 20.46, p<0.0001) and Dunnett's multiple comparisons. P-values represented by symbols as follows: p<0.05 (*), p<0.01 (**), p<0.001 (***), P<0.0001 (****). Percentage change in dye extravasation relative to vehicle treated animals is indicated by the number adjacent to the pair-wise comparison lines.

CONCLUSIONS

- THB001 is a highly potent and selective small molecule KIT inhibitor as determined in several biochemical and cell-based assays
- THB001 displayed approximately 50-fold or greater selectivity against key anti-kinase targets CSF-1R and PDGFRβ
- THB001 has high oral exposure reaching sufficient free drug plasma levels to surpass the *in vitro* calculated IC_{50} levels
- Daily oral dosing of THB001 in rats results in a statistically significant and dose dependent depletion of skin mast cells within the ears of treated animals
- THB001 treatment prevented the mast cell driven, anaphylaxis mediated extravasation of Evans Blue dye in the rat PCA model

DISCLOSURES

T. Thullen, B.T. Chamberlain, C. Kent, G. Keaney, and A.S. Ray are current employees of Third Harmonic Bio.

G.C. Parry and S. Yoo are former employees of Third Harmonic Bio.

REFERENCES

[1] Steven P. Sweeney, Christine Voors-Pette, Jerome Oude Nijhuis, Gregg Keaney, Graham Parry, Edward Conner, Darrell Nix, Nathalie Rioux, Amy DiRico, Stephen Yoo - First-in-human Safety, Pharmacokinetics and Pharmacodynamics of THB001: An Orally Available, Potent and Highly Selective Small Molecule Inhibitor of Wild Type KIT Receptor Tyrosine Kinase. AAAAI Annual Meeting; February 2023. **Poster 156**

[2] Davis, MI, Zarrinkkar, PP, *et al*. Comprehensive Analysis of Kinase Inhibitor Selectivity. *Nature Biotechnology* 2011; 29: 1046-1051.

[3] Machleidt T, Wood KV *et al.* NanoBRET – A Novel BRET Platform for the Analysis of Protein-Protein Interactions. *ACS Chem Biol.* 2015; 10(8): 1797-1804