Discovery and Characterization of Orally Bioavailable Nonpeptide Thyroid Stimulating Hormone Receptor (TSHR) Antagonists for the Treatment of Graves' Disease

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Graves' disease is an autoimmune condition that affects approximately 1 in 100 people in the United States and 2-3% of the population worldwide. It is characterized by the production of autoantibodies against TSHR, and the pathology of Graves' disease is driven by TSHR stimulatory antibodies (TSAb) that result in heightened activation of TSHR. This overstimulation results in hyperthyroidism due to excessive production of thyroid hormones. Approximately 30% of Graves' disease patients also develop thyroid eye disease (TED or Graves' orbitopathy) due to overactivation of TSHR in orbital fibroblasts leading to excessive production of hyaluronic acid, adipogenesis, cytokine production, and fibrosis. This can cause a myriad of debilitating symptoms including pain, swelling, blurry vision, diplopia, and proptosis. Several treatments for Graves' hyperthyroidism are available including anti-thyroid drugs, radioactive iodine (RAI), and surgery. RAI and surgery are definitive treatments for Graves' hyperthyroidism, but often result in hypothyroidism. In addition, none of the current treatments for Graves' hyperthyroidism are effective in treating TED and, in some cases, such as with RAI, worsen the condition. Blocking TSHR activation directly via a TSHR antagonist may provide an important new therapeutic mechanism to treat patients with Graves' disease that would effectively treat both the hyperthyroidism and TED.

Crinetics has identified several potent and orally bioavailable nonpeptide allosteric antagonists with acceptable drug-like properties. One analog, TSHRant-1, demonstrated potent negative allosteric modulator activity at both the human and rat TSHR. To evaluate the in vivo pharmacodynamics of TSHant-1, we developed a rat model of hyperthyroidism. In this model, subcutaneous administration of the TSAb, M22, to female rats resulted in a robust and long-lasting rise in levels of the thyroid hormone thyroxine (T4). Oral administration of TSHRant-1 dose-dependently suppressed M22-stimulated T4, providing evidence that a nonpeptide allosteric antagonist of TSHR may serve as an effective treatment for Graves' disease and associated orbitopathy (TED).

Hypothesis: A TSHR Antagonist Could Serve as a Treatment for Graves' Hyperthyroidism and TED

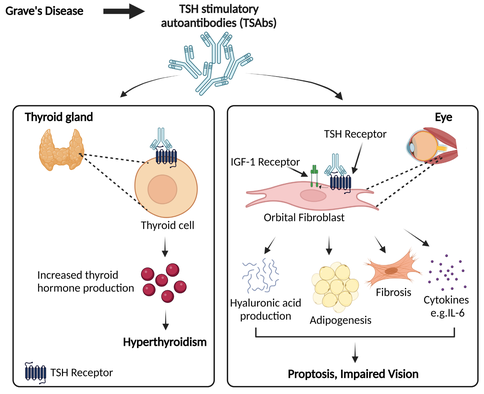

Figure 1. Pathophysiology of Graves' Hyperthyroidism and Graves' Orbitopathy/Thyroid Eye Disease (TED). Stimulatory autoantibodies (TSAbs) generated against TSHR cause inappropriate activation of TSHR in the thyroid and orbital fibroblasts, resulting in uncontrolled production of thyroid hormones and increased hyaluronic acid production, adipogenesis, and fibrosis in the eye. TSHR antagonists could block the action of TSAbs in the thyroid and orbital fibroblasts. Created with BioRender.com.

TSHR Antagonist Assay Cascade

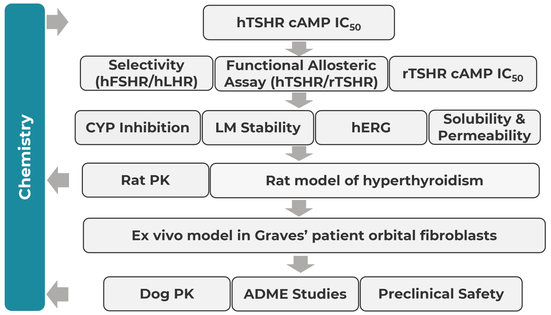

Figure 2. Assay cascade to identify TSHR antagonists. Compounds are screened in TSHR cAMP assays to identify potent TSHR antagonists, which are then screened against related glycoprotein hormone receptors FSHR and LHR. Drug-like properties are assessed by screening for hERG channel and CYP inhibition, liver microsomal (LM) stability, solubility, and permeability. Suitable molecules are evaluated in a rat model of hyperthyroidism and ex vivo in orbital fibroblasts obtained from Graves' patients. A subset of these molecules are evaluated for ADME properties and preclinical safety.

TSHant-1 Is Potent in Functional Antagonist Assays Targeting Human and Rat TSHR

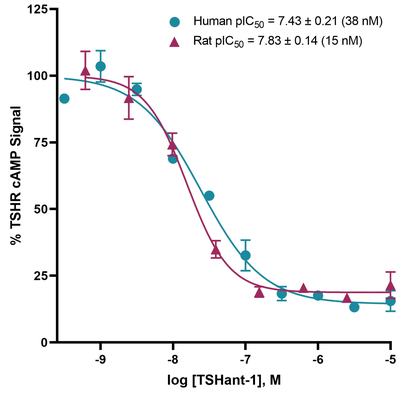

Figure 3. Functional antagonist assay at human and rat TSHR. Cells heterologously expressing human or rat TSHR were treated with an EC_{80} concentration of the antibody agonist M22 in the presence of multiple concentrations of TSHant-1. cAMP production was quantified after a 30-minute incubation, and IC_{50}s were calculated from concentration-response curves. TSHant-1 had a max inhibition of 80% in both assays. Points represent mean ± SEM of three technical replicates. Curves are representative of ≥2 independent experiments.

TSHant-1 Is an Allosteric Antagonist that Decreases Efficacy of TSHR Stimulatory Antibody, M22

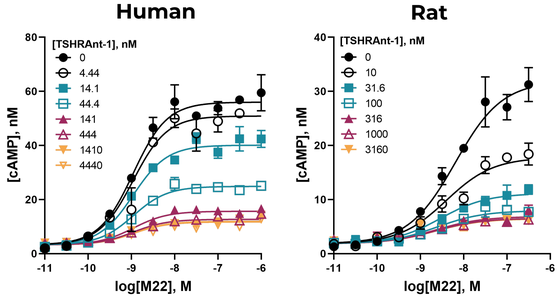

Figure 4. Effects of TSHant-1 on M22 concentration response curves in human TSHR expressing (left) and rat TSHR expressing (right) cell lines. The results are consistent with a noncompetitive, allosteric mechanism, where TSHRant-1 decreases the efficacy of the antibody agonist at the receptors. Human pK_B = 7.49 ± 0.16 (33 nM), Rat pK_B = 7.78 ± 0.12 (17 nM). Points represent mean ± SEM of two data points. Graphs are representative of ≥3 independent experiments.

M22-Stimulated T4 in Rats Serves as a Preclinical Model of Hyperthyroidism

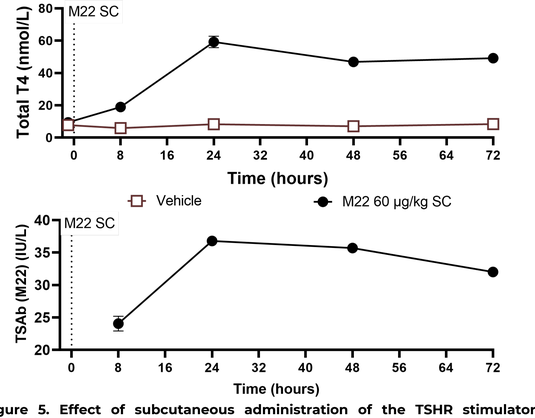

Figure 5. Effect of subcutaneous administration of the TSHR stimulatory antibody, M22, on T4 levels in rats. Five-week-old female Sprague Dawley rats received a single subcutaneous administration of 60 µg/kg/day M22 to induce a rise in serum total T4 (top panel).T4 levels began to rise at 4h post dose and remained elevated up to 72h post M22 administration. Serum levels of M22 (bottom) peaked at 24h post dose and remained elevated up to 72h post dose. Points represent mean ± SEM (n= 8 rats/group).

TSHant-1 Suppresses M22-Stimulated T4 in Rats

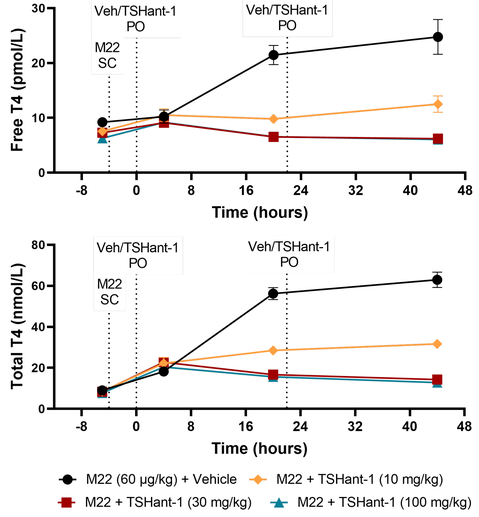

Figure 6. Effect of oral administration of TSHant-1 on M22-stimulated total and free T4 levels in rats. Five-week-old female Sprague Dawley rats received a single subcutaneous administration of 60 µg/kg/day M22 to induce a rise in serum free T4 (top panel) and total T4 (bottom panel). Four hours post M22 administration (t=0h), TSHant-1 was administered once daily by oral gavage for 2 days. Free and total T4 levels were measured at -5h, 2h, and 4h post dose on Day 1, and -2h and 24h post dose on Day 2. Points represent mean ± SEM (n=8 rats/group).

CONCLUSIONS

Crinetics has identified a potent and orally bioavailable nonpeptide allosteric antagonist of TSHR that suppresses M22-stimulated T4 in a rat model of hyperthyroidism.

These studies provide evidence that nonpeptide antagonists of TSHR may serve as an effective treatment for Graves' disease.

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