Blocking Activation of the Parathyroid Hormone Receptor Type 1 (PTH1R) with a Specific and Orally Available Antagonist is a Promising Therapy for the Treatment of Primary Hyperparathyroidism (PHPT)

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BACKGROUND AND HYPOTHESIS

Primary hyperparathyroidism (PHPT) is a disorder caused by the enlargement and overactivation of one or more of the parathyroid glands resulting in over-secretion of PTH and high levels of calcium in blood. PHPT affects approximately 100,000 patients per year and, for those who are symptomatic (~20%) there are several complications in the skeletal, renal, gastrointestinal, and neurological systems, such as osteoporosis, kidney stones, loss of appetite, fatigue, and confusion, among others. Parathyroidectomy is first-line treatment, but for patients with recurring symptoms or the inability to have surgery, medical therapy is required. However, current therapies are limited and do not fully restore calcium homeostasis or completely alleviate the complications associated with the disorder. We hypothesize that blocking PTH action via a PTH receptor antagonist will provide an improved and novel therapeutic mechanism to treat PHPT patients.

Hypercalcemia Associated With Primary Hyperparathyroidism

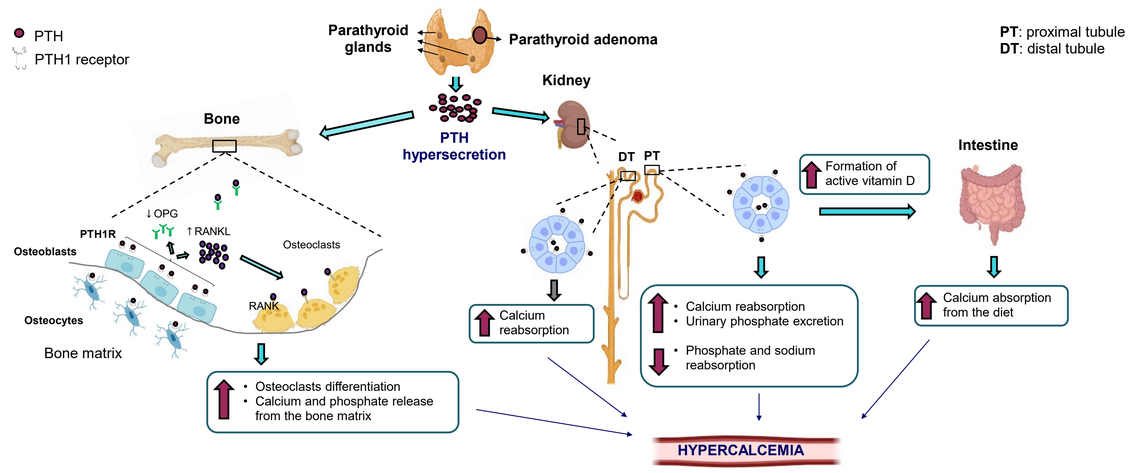
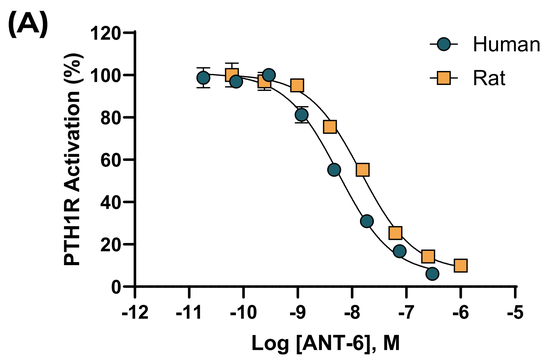


Figure 1. PTH over secretion hyperactivates PTH1R expressed in the target tissues (bone and kidney) augmenting the normal PTH actions and ultimately resulting in changes of important modulators of calcium homeostasis and hypercalcemia.

RESULTS

PTH1R Antagonist ANT-6 prevents the binding and activation of PTH1R by PTH(1-34) across species



PTH1R	IC_{50}, nM	pIC_{50} ± SEM
Human	4.1	8.4 ± 0.1
Monkey	11	7.9 ± 0.1
Dog	1.6	8.8 ± 0.1
Rat	9.5	8.0 ± 0.0
Mouse	42	7.4 ± 0.1

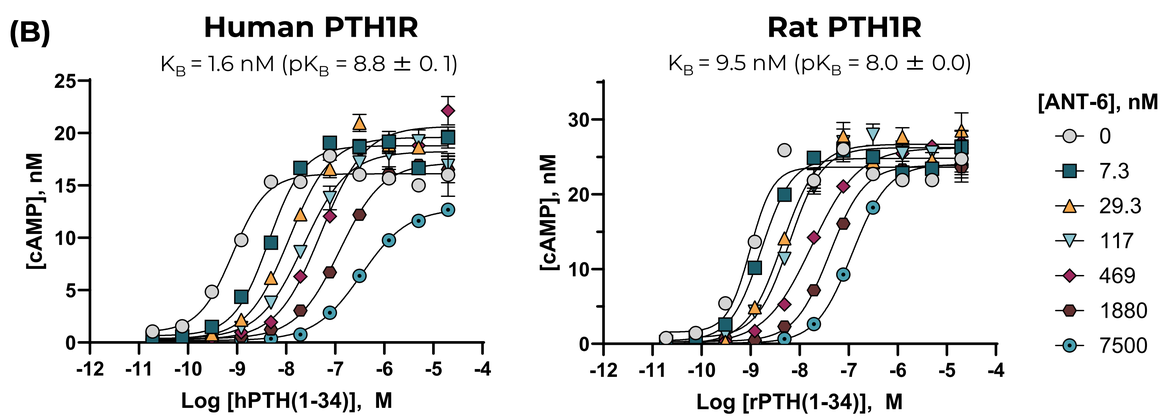
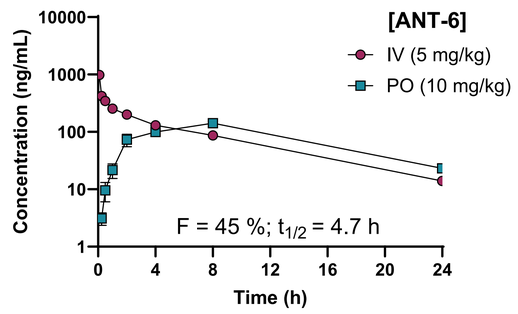


Figure 2. (A) Antagonist assay in cells expressing PTH1R from several species. Cells were treated with ANT-6 in the presence of a constant concentration of agonist (human or rat PTH(1-34)). cAMP production was quantified after 0.5h incubation, and IC_{50}s were calculated from concentration-response curves. ANT-6 potencies at all receptors evaluated are shown in the table. **(B) Allosteric assay.** Cells were treated with agonist (human or rat PTH(1-34)) and increasing concentrations of ANT-6. cAMP production was measured after 1h incubation, and K_B (affinity constant) was calculated from the concentration-response curves. Graphs are representative of two independent experiments and each point is the mean ± SEM of three technical replicates.

RESULTS (cont.)

ANT-6 is orally bioavailable and has good drug-like properties



Property	ANT-6
CYP2D6 inhibition, μM	> 10
CYP3A4 inhibition, μM	> 8.6
hERG inhibition, μM	6.7
Oral bioavailability-F (rat/dog), %	45 / 69
Half-life t½, (rat/dog), h	4.7 / 22

Figure 3. ANT-6 pharmacokinetic plasma profile in rat and drug-like properties. Sprague Dawley rats were administered ANT-6 at 5 mg/kg (IV-intravenous) and 10 mg/kg (PO-oral). ANT-6 concentration was measured in plasma. Points represent mean ± SEM (n=3). Table shows ANT-6 screened for CYP and hERG inhibition, and oral bioavailability in rat and dog.

ANT-6 blocks PTH-dependent changes in body weight and ionized calcium in a rat model of hypercalcemia

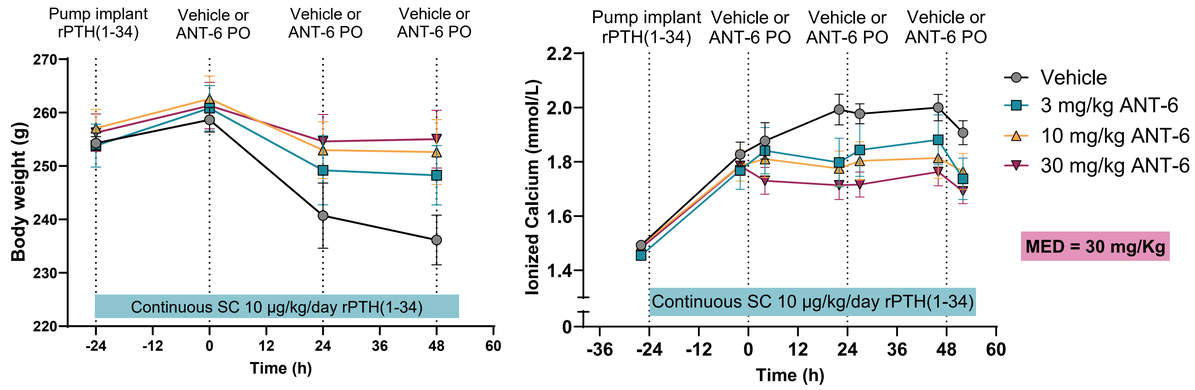


Figure 4. Effect of oral administration of ANT-6 in a rat model of hypercalcemia. Adult male Sprague Dawley rats received continuous SC rat PTH(1-34) (10 μg/kg/day) to induce hypercalcemia. Starting 24h post pump implant, ANT-6 was administered once daily by oral gavage for 3 days and blood ionized calcium was measured at -2h and 4h post dose. Points represent mean ± SEM (n=7-8 rats/group). Minimum efficacious dose (MED) was determined based on the statistically significant difference in comparison to vehicle

ANT-6 blocks PTH1R activation and PTH-dependent changes in RANKL and OPG mRNA expression in human primary osteoblasts

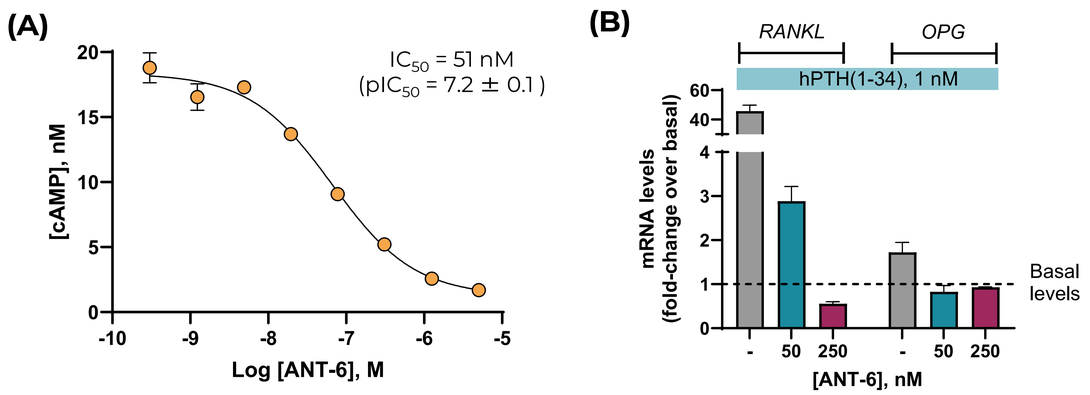


Figure 5. (A) Antagonist assay in human primary osteoblasts. Differentiated cells were stimulated with 3.5 nM hPTH(1-34) (~EC_{80}) and ANT-6 for 1h followed by cAMP quantification. ANT-6 potency was calculated from the concentration-response curve and expressed as IC_{50}. Graph is a representative of 3 independent experiments and shows mean ± SEM of 3 technical replicates. **(B) Changes in gene expression of RANKL and OPG.** Differentiated cells were treated for 8h with hPTH(1-34) and ANT-6. mRNA levels were measured by real-time RT-PCR and normalized to *β-actin*. Dotted line indicates the relative expression of cells treated with vehicle and the grey bar represents cells receiving hPTH(1-34) only. Bars represent mean ± SEM of 3 technical replicates.

CONCLUSION

ANT-6 is shown to block the actions of PTH at the receptor level, in a pharmacodynamic model of hypercalcemia in rats, and in human primary osteoblasts. These data support the idea that ANT-6 may ameliorate hypercalcemia and improve bone homeostasis in patients with PHPT

ACKNOWLEDGMENTS
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DISCLOSURES
All authors are employees of Crinetics Pharmaceuticals, Inc.