Effects of an Orally Bioavailable Nonpeptide Parathyroid Hormone Receptor Type 1 (PTH1R) Antagonist in Rodent Models of Primary Hyperparathyroidism (PHPT)

Agnes S. Antwan, Elizabeth Rico-Bautista, Joseph Pontillo, Shimiao Wang, Michael A .Johns, Bastian Ramms, Melissa A. Fowler, Julie B. Nguyen, Anel A. Castellanos Gonzalez, Beth Fleck, Kelsey Retting, Deepak Dalvie, Stephen F. Betz and Stacy Markison
Crinetics Pharmaceuticals, San Diego, CA.

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Primary hyperparathyroidism (PHPT) is a condition resulting from an over-secretion of parathyroid hormone (PTH) from one or more overactive parathyroid glands. PTH is a peptide hormone that regulates blood calcium concentrations through effects on bone, intestines, and kidney. PTH acts on PTH1R in the kidney to increase calcium reabsorption and block phosphate reabsorption. The loss of phosphate ions then causes an increase in ionized calcium in the blood and cAMP in the urine. At the bone, continuous PTH infusion stimulates osteoclast activity while inhibiting osteoblast activity, leading to breakdown of bone and release of calcium into the extracellular fluid. Conditions that cause excess PTH, such as PHPT, result in elevated concentrations of plasma calcium, increased bone loss, increased fracture risk, and higher susceptibility to kidney stone development. PHPT affects approximately 100,000 patients per year, but many patients are asymptomatic and remain undiagnosed. While a partial or total parathyroidectomy may be suitable for patients diagnosed with severe cases of PHPT, there are limited treatment options for those who do not meet the criteria or do not wish to undergo surgery. The goals for medicinal treatment are to normalize blood calcium levels and urinary calcium excretion, as well as increase bone mineral density to reduce fracture risk. Blocking PTH action directly via a potent PTH1R antagonist may provide an important new therapeutic mechanism to treat patients with PHPT.

Using iterative medicinal chemistry and pharmacology, Crinetics has identified several potent and orally bioavailable PTH1R antagonists with good drug-like properties. Lead molecules were evaluated in vivo in preclinical rodent models for their ability to suppress the effects of excess PTH on serum calcium, bone turnover, and cAMP levels in the kidney.

Targeting PTH1R could improve conditions associated with PTH over-secretion

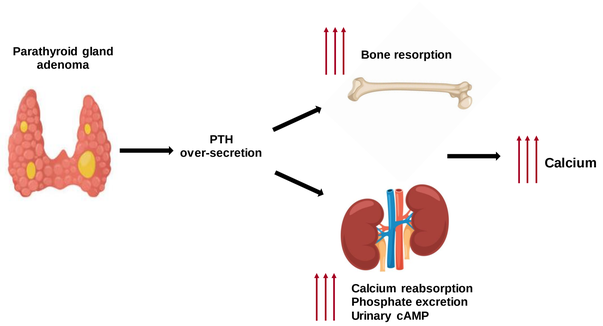

Figure 1. Primary Hyperparathyroidism associated with PTH over-secretion. PTH over-secretion due to a parathyroid gland adenoma will hyperactivate PTH1R expressed in the bone and kidney inducing changes of important modulators of calcium homeostasis.

ANT-5 behaves as an allosteric modulator at PTH1R

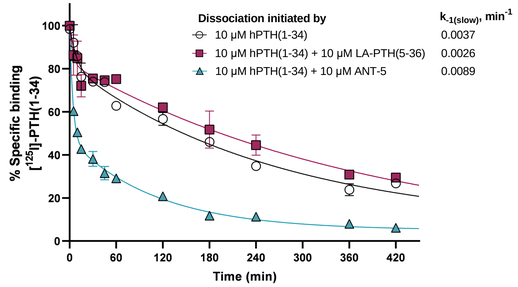

Figure 2. ANT-5 significantly affects the dissociation of hPTH(1-34) from hPTH1R, indicating an allosteric interaction. [125I]-hPTH(1-34) was allowed to bind Chem-1 cell membranes heterologously expressing hPTH1R. Dissociation was best described by two phases with 20% of binding dissociating at the rapid rate. The presence of the orthosteric antagonist LA-PTH(5-36) did not change the dissociation of [125I]-PTH(1-34). In contrast, ANT-5 significantly increased the rate of the slow dissociation phase (table above) and increased the % binding in the rapid phase to 56% (GraphPad Prism sum-of-squares F test).

ANT-5 decreases affinity and efficacy of PTH

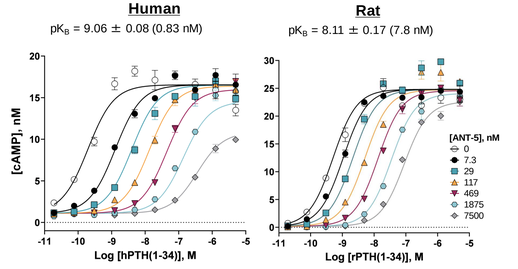

Figure 3. Effects of ANT-5 on peptide agonist concentration response curves in human and rat PTH1R expressing cell lines. The results are consistent with a noncompetitive, allosteric mechanism, where ANT-5 decreases the affinity and efficacy of the agonists for the receptors.

ANT-5 has good drug-like properties and is orally bioavailable

	CYP450 Inhibition (µM)		hERG (µM)	CLint (mL/min/kg)		Rat PK		Dog PK	
	2D6	3A4		Human	Rat	F%	$t_{½}$ (h)	F%	$t_{½}$ (h)
ANT-5	5.7	8.3	6.8	1.4	14.8	38	5.7	45	32

Table 1. Drug-like characteristics of ANT-5. ANT-5 was screened for CYP and hERG inhibition, liver microsomal (LM) stability, and oral bioavailability in rat and dog.

ANT-5 suppresses ionized calcium in a rat model of PTH-induced hypercalcemia

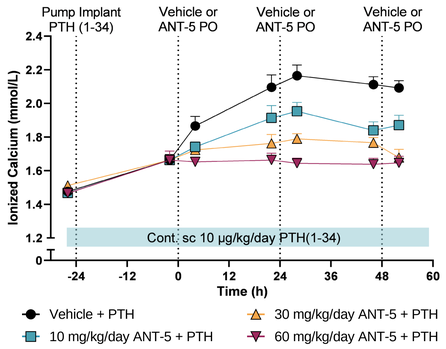

Figure 4. Effect of oral administration of ANT-5 on ionized calcium levels in rat PTH-dependent model of hypercalcemia. Adult male Sprague Dawley rats received continuous subcutaneous infusion of 10 µg/kg/day rat PTH(1-34) via osmotic minipump to induce a rise in blood ionized calcium. Starting at 24 hours post pump implant, ANT-5 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).

ANT-5 suppresses PTH-stimulated increase of urinary cAMP in rats

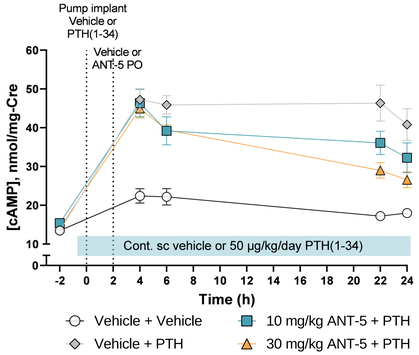

Figure 5. Effect of oral administration of ANT-5 on PTH-induced urinary cAMP. Adult male Sprague Dawley rats received continuous subcutaneous infusion of vehicle or 50 µg/kg/day rat PTH(1-34) via osmotic minipump to induce a rise in urinary cAMP. Starting at 2 hours post pump implant, ANT-5 was administered by oral gavage and urinary cAMP was measured at -2h, 4h, 6h, 20h, and 22h post dose. Points represent mean ± SEM (n=7-8 rats/group).

ANT-5 suppresses bone resorption in a rat model of PTH-induced bone turnover

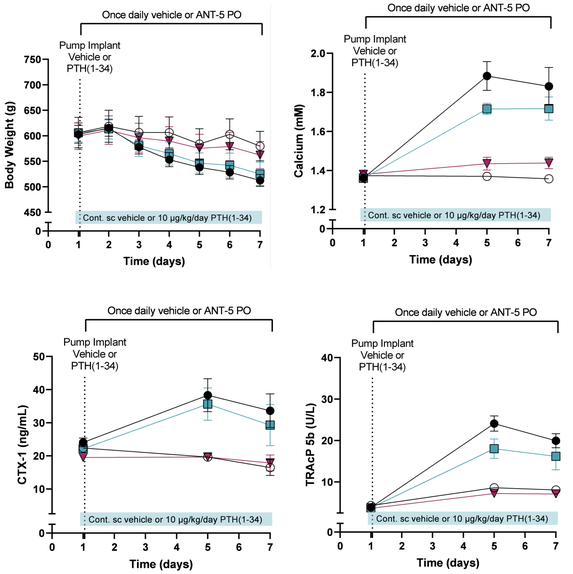

Figure 6. Effect of oral administration of ANT-5 on bone resorption in rat PTH-dependent model of bone turnover. Adult male Sprague Dawley rats received continuous subcutaneous infusion of vehicle or 10 µg/kg/day rat PTH(1-34) via osmotic minipump to induce bone turnover. Starting at 2 hours post pump implant, ANT-5 was administered once daily by oral gavage for 7 days. Bone resorption was measured on days 1, 5, and 7. Points represent mean ± SEM (n=7-8 rats/group).

Conclusion

Crinetics has developed a potent, drug-like PTH1R negative allosteric modulator that suppresses PTH-stimulated increases in ionized calcium, urinary cAMP, and bone resorption biomarkers in rats and could be a viable treatment for Primary Hyperparathyroidism in humans.

Crinetics Pharmaceuticals