SAT 339

A Novel anti-PTH1R Receptor Antagonist Monoclonal Antibody Reverses Hypercalcemia Induced by PTH or PTHrP: a Potential Treatment of Primary Hyperparathyroidism and Humoral Hypercalcemia of Malignancy

Agnes Choppin, Daniel Bedinger, Rachel Hunt, Catarina Tran, Sujeewa Wijesuriya, Robyn Cotter, Elizabeth Pongo, Robert Shimizu, Jessica Chen, Khanh Pham, Amer Mirza, Ou Li, Kirk Johnson*, Toshihiko Takeuchi, Raphael Levy

XOMA Corporation, 2910 Seventh Street, Berkeley, CA 94710

ABSTRACT

The PTH1R receptor is one of the family B GPCRs and the primary receptor of two ligands, parathyroid hormone (PTH) and parathyroid related protein (PTHrP). Hypercalcemia can occur when elevated levels of PTH, as seen in primary hyperparathyroidism (PHPT), or elevated levels of PTHrP, as seen in humoral hypercalcemia of malignancy (HHM), leading to excessive activation of the PTH1R receptor. A potent and long acting receptor antagonist could reverse hypercalcemia in these conditions. We therefore have developed a highly potent PTH1R antagonist monoclonal antibody for the treatment of disorders related to elevated PTH or PTHrP. This antibody was discovered using fully human phage display libraries and selected for potent binding to and antagonism of the human and murine PTH1R. PTH1R antagonism by our antibody against both the PTH and PTHrP peptides was determined by cAMP accumulation in osteosarcoma cell lines Saos-2 (human) and UMR106 (rat). The antibody showed roughly equivalent inhibition of both the human and murine receptors. We also demonstrated in vitro that this antibody inhibited both PTH- and PTHrP-induced osteoclast differentiation by greater than 10-fold in a Saos-2 and human monocyte co-culture system: the PTH1R receptor is expressed on osteoblasts and osteocytes, and stimulation by PTH or PTHrP leads these cells to increase the expression of RANKL and other factors. These drive the differentiation and activation of bone resorbing osteoclast cells, which release calcium and decrease bone density. In vivo proof-of-concept was obtained in rodent models where hypercalcemia was established in rats by SC infusion of PTH or PTHrP via osmotic pumps. IV administration of 2 and 10 mg/kg antibody dose-dependently reduced serum calcium levels by a minimum of 2 mg/dL within 48 hours of dosing. Additionally, the antibody was tested in a model of HHM wherein mice developed hypercalcemia following implantation of mouse colon tumor cells C26. In this tumor model, the antibody given at 10 mg/kg IV was capable of completely reversing hypercalcemia within 24 hours. The pharmacokinetic parameters of the antibody were also defined in rats.

This highly potent PTH1R receptor antagonist antibody has the potential to become a valuable therapeutic agent in a variety of indications including hyperparathyroidism, humoral hypercalcemia of malignancy, and, potentially, the PTHrP-mediated cachexia seen in some cancers.

INTRODUCTION

XOMA PTH1R Antibodies	Potential first-in-class therapeutic with MOA designed to inhibit PTH1R Acts upstream of RANKL - addresses bone & kidney related hypercalcemia Targets the receptor (PTH1R) rather than the ligand (PTH, PTHrP) to potentially offer sustained normalization of serum calcium and prevent compensation with high levels of PTH, PTHrP
Lead indications	**Humoral Hypercalcemia of Malignancy (HHM):** Current treatments have limited efficacy or severe side effect profile **Primary Hyperparathyroidism (PHPT):** Need for effective medical treatment for 5-10% of Primary Hyperparathyroidism (PHPT) patients who fail or can't undergo surgery
Additional Potential Indications	**Parathyroid Carcinoma (PC):** Ultra-orphan, but high unmet need: 50% fail parathyroidectomy and only approved drug (cinacalcet) often used at poorly-tolerated doses. **Secondary HPT:** Majority of ESRD patients develop; Poor response and tolerability to cinacalcet. **Metastatic Bone Disease:** High prevalence and PTHrP is likely critical. Single agent or combo.
Program Status	Demonstrated in vivo POC in rat models with PTH or PTHrP-driven hypercalcemia and mouse tumor models for HHM. Development candidate selected. Ready for development to IND

Activity Profile of anti-PTH1R mAb(s)

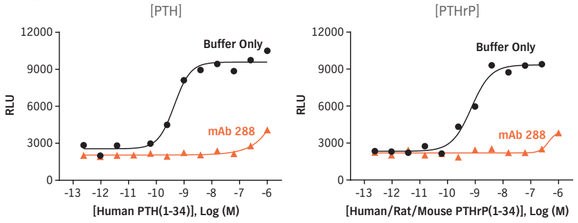
Property	Fully Human mAb to the Human Parathyroid Hormone Receptor
Affinity (K_D)	High selectivity lead antibody with high affinity (K_D ~0.2 nM)
In vitro Potency	Observed near complete reduction of cAMP with both PTH and PTHrP ligands against human and rodent PTH1R-expressing cell lines
In vivo Proof of Concept	Observed a greater than 2.5 mg/dL reduction of PTHrP- or PTH-induced calcium with therapeutic dosing as low as 2 mg/kg
Effective Dose Range and Administration	Substantial serum calcium reduction observed from 2 to 10 mg/kg in animals Subcutaneous administration every 4 weeks anticipated in the clinic
Species Cross-Reactivity	Demonstrated cross-reactivity with similar potency to human, rodent, and monkey PTH1R

Description of Lead anti-PTH1R mAbs

Anti-PTH1R mAb	Descriptor	Comment
288	IgG2 in vitro & in vivo advanced lead	KD 0.2 nM; IC_{50} 5.2 nM Affinity-matured from mAb 012 lead candidate (20 nM)
349	IgG2 Development Candidate	KD 0.2 nM ; IC_{50} 5.7 nM Codon-optimized for efficient manufacturing
353	IgG4 development option	KD 0.2 nM ; IC_{50} 6.2 nM Improved cell expression vs 349 Most recent scale-up

RESULTS

Inhibition of PTH- and PTHrP-Induced cAMP in SaOS-2 Cells Expressing Native Human PTH1R

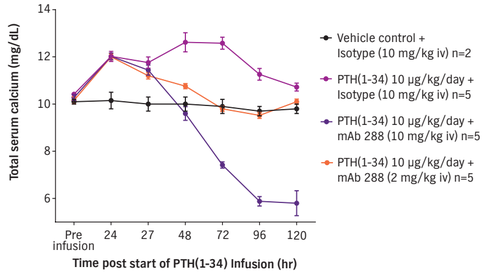


Anti-PTH1R mAb Efficacy in Rat Models of Primary Hyperparathyroidism or Hypercalcemia due to PTH or PTHrP

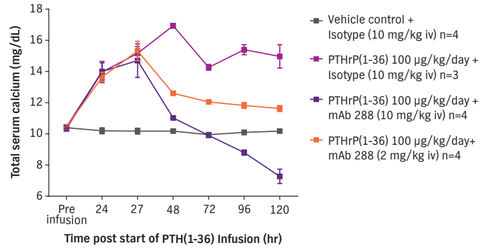
Normal SD rats infused with human PTH or PTHrP via subcutaneous osmotic pumps

- Study design
  - 5 days sc infusion (Alzet mini pumps)
    - hPTH(1-34) 10 µg/kg/day
    - PTHrP 100 µg/kg/day
  - Isotype (10 mg/kg IV), mAb 288 (2 or 10 mg/kg IV) dosed 24 hr post PTH infusion (ΔCa is increased ~2 mg/dL from baseline)
  - Serum collection for Ca & other potential markers

mAb 288 Potently Reduced PTH-Induced Hypercalcemia in Rats



mAb 288 Potently Reduced PTHrP-Induced Hypercalcemia in Rats

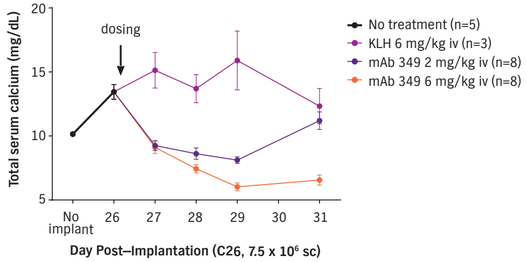


Validating anti-PTH1R mAbs in Mouse Models for HHM: Tumor growth – PTHrP elevation – Hypercalcemia

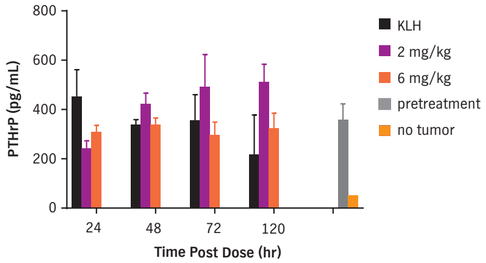
Pilot studies validated with 3 tumor models:

- Study design
  - mouse colon C26
  - human prostate cancer PC-3
  - human lung cancer HARA-B
- Monitor s.c. tumor growth and serum Calcium and administer anti-PTH1R mAb when hypercalcemic.
- Endpoints = total serum Ca and plasma (and serum) PTHrP levels

PTH1R mAb 349 Potently Reduces Mouse Colon 26 tumor-related Hypercalcemia for a Sustained Period



Serum PTHrP is elevated in tumor-bearing mice. Anti-PTH1R mAb 349 did not significantly change PTHrP levels, but inhibited its action



SUMMARY

- A high affinity fully human mAb to PTH1R has been selected and characterized.
- Efficacious in rodent models of primary hyperparathyroidism or hypercalcemia of malignancy
  - ED_{50} < 2 mg/kg
  - Duration of action ~5 days at lowest tested dose. Anticipated increase of antibody half-life in humans, as we've typically observed, should result in a substantially longer duration of action.
- Lead candidate ready for development as a novel, First-in-Class therapeutic