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

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ABSTRACT

The PTH1R antagonist is one of the first G GPCRs and the primary receptor of two ligands, parathyroid hormone (PTH) and parathyroid hormone-related protein (PTHR). 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), to excessive activation of the PTH1R receptor. A potent and long acting receptor antagonist could reverse hypercalcemia in those conditions. We have developed a highly potent PTH1R monoclonal antibody (mAb) that reverses the symptoms of disorders related to elevated PTH or PTHR. This antibody was discovered using fully human phage display libraries and selected for potent binding to and activation of the human and murine PTH1R. PTH1R antagonism by our antibody against both the PTH and PTHrP peptides was determined by cAMP accumulation in osteoclast cell lines (Saos-2) and (RAW264.7). The antibody showed roughly equivalent efficacy in both the human and murine receptors. We also demonstrated in vitro that the antibody inhibited both PTH and PTHR-induced calcium efflux by greater than 10-fold in a Saos-2/human osteoclast-like system. The PTH1R antibody 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 osteoclasts, which release calcium and increase bone density. In vivo proof-of-concept was obtained in rodent models where hypercalcemia was established in rats by s.c. infusion of PTH or PTHrP via osmotic pumps. In administration of 2 or 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 were given hPTH (1-34) i.p. Serum PTHrP is elevated in tumor-bearing mice. Anti-PTH1R mAb 349 did not significantly change PTHrP levels, but inhibited its action.

INTRODUCTION

Primary hyperparathyroidism (PHPT) is a common cause of hypercalcemia that can be life-threatening if left untreated. It is characterized by excessive production of parathyroid hormone (PTH), which stimulates bone resorption and renal calcium reabsorption, leading to increased serum calcium levels. Hypercalcemia can cause a range of symptoms, including bone pain, kidney stones, and increased risk of cardiovascular disease. The current treatment for PHPT is surgical parathyroidectomy, but it is not always successful, and there is a need for new therapeutic options.

The PTH1R antagonist mAb 349 is a fully human monoclonal antibody that selectively targets the PTH1R receptor. It is designed to inhibit the binding of PTH and PTHrP to the PTH1R, thereby reducing the downstream effects of these hormones. The mAb 349 has demonstrated potent in vitro and in vivo activity in various models of hypercalcemia.

METHODS

**In Vitro Activity**

The mAb 349 was tested for its ability to inhibit PTH and PTHrP-induced cAMP accumulation in Saos-2 and RAW264.7 cell lines. The antibody showed comparable inhibition of both human and murine PTH1R receptors. The mAb 349 also demonstrated equivalent inhibition of both human and murine PTH1R-expressing cell lines.

**In Vivo Activity**

The mAb 349 was tested in vivo in rodent models where hypercalcemia was established by s.c. infusion of PTH or PTHrP via osmotic pumps. In administration of 2 or 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 were given hPTH (1-34) i.p. Serum PTHrP is elevated in tumor-bearing mice. Anti-PTH1R mAb 349 did not significantly change PTHrP levels, but inhibited its action.

**Cross-Reactivity**

The mAb 349 demonstrated cross-reactivity with similar potency to human, rodent, and monkey PTH1R receptors. It showed roughly equivalent inhibition of both human and murine PTH1R-expressing cell lines.

**Pharmacokinetics**

The mAb 349 was administered subcutaneously every 4 weeks, and substantial serum calcium reduction was observed from 2 to 10 mg/kg in rodents. It was also tested in a model of HHM wherein mice were given hPTH (1-34) i.p. Serum PTHrP is elevated in tumor-bearing mice. Anti-PTH1R mAb 349 did not significantly change PTHrP levels, but inhibited its action.

**Target Engagement**

The mAb 349 selectively inhibited PTH- and PTHrP-induced hypercalcemia in rats. It 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 were given hPTH (1-34) i.p. Serum PTHrP is elevated in tumor-bearing mice. Anti-PTH1R mAb 349 did not significantly change PTHrP levels, but inhibited its action.

**Clinical Trials**

The mAb 349 is currently in clinical development, with Phase 2 trials planned for both PHPT and HHM. It is expected to be well tolerated and have a favorable safety profile.

**Conclusion**

The mAb 349 is a promising therapeutic option for the treatment of PHPT and HHM. Its potent in vitro and in vivo activity, combined with its good safety profile, makes it a promising candidate for further clinical development.