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Why target the parathyroid calcium-sensing receptor for the treatment of secondary hyperparathyroidism?

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ABSTRACT

Secondary hyperparathyroidism (HPTH-II) is a common and serious complication of chronic kidney disease (CKD). It affects more than 300 000 end-stage renal disease patients treated by dialysis and probably more than three millions patients with CKD worldwide. It is associated with an increased risk of cardiovascular calcifications and mortality. The traditional therapies to lower serum parathyroid hormone (PTH) levels and to control HPTH-II consisted of calcium salts and vitamin D. However, the draw-back of these therapies is a resulting hypercalcemia, hyperphosphatemia, and lack of long-term efficacy. On the other hand, the surgical treatment of HPTH-II by total or subtotal parathyroidectomy is a radical therapeutical option which is not exempt of complication notably a permanent state of hypoparathyroidism which in many cases is responsible of an increased serum calcium phosphorus product and soft tissue calcifications. The identification of the parathyroid calcium-sensing receptor (CaR) has led to the development of calcimimetics, a new class of drugs able to reduce the serum PTH concentration by acting directly at the CaR level (or "on the CaR"). This article reviews the main reasons that led to target this CaR for the treatment of HPTH-II: i) The activation of the CaR by small changes in extracellular ionized calcium is the major regulator of PTH secretion, ii) inactivating mutations in the CaR gene are responsible of two parathyroid gland hyperfunctioning states as in familial

hypocalciuric hypercalcemia and neonatal severe hypercalcemia, iii) the activation of the CaR by a high calcium containing diet prevents the parathyroid hyperplasia seen in VDR null mice, iv) the parathyroid hyperplasia seen in uremic animals can be stopped or prevented by the administration of CaR agonists, v) the reduced expression of the parathyroid CaR seen in uremic states can be corrected by the CaR agonists, and vi) the activation of the CaR by the calcimimetics allows to significantly reduce serum PTH levels in dialysis patients with HPTH-II. In summary, targeting the CaR function is the only way to specifically decrease PTH secretion without increasing serum calcium phosphorus product.

Key words: Calcimimetics, Parathyroid hormone, Hemodialysis, Renal osteodystrophy, Calcium

INTRODUCTION

Secondary hyperparathyroidism (HPTH-II) is a common complication in patients with chronic kidney disease (CKD). It affects more than 300 000 end-stage renal disease patients treated by dialysis and probably more than three millions patients with CKD[1]. It is associated with an increased risk of cardiovascular calcifications and mortality[2]. Although surgical parathyroidectomy (PTX) remains the gold standard therapy, it is not without risk. PTX exposes to anesthesia risks, surgical complications, and permanent hypoparathyroidism[3]. On the other hand, traditional therapies such as calcium salts and vitamin D are limited by a resulting hypercalcemia, hyperphosphatemia, and lack of long-term efficacy. The new class of drugs, called calcimimetics, amplify the sensitivity of the parathyroid calcium-sensing receptor (CaR) to calcium, thereby reducing serum PTH concentration[4,5]. This paper reviews the principal reasons for targeting the parathyroid CaR in the treatment of HPTH-II.

REGULATION OF THE PTH SECRETION BY THE CALCIUM "SET-POINT"

The CaR from the parathyroid gland is the principal regulator of PTH secretion. When serum calcium decreases, the CaR is inhibited and PTH-containing vesicles move to the cell membrane and release PTH to the circulation. When serum calcium increases, the CaR is activated and the release of PTH is inhibited. There is a sigmoid relationship between the secretion of PTH and the serum calcium concentration which led to the definition of a calcium "set-point" which is the ionized serum calcium concentration corresponding to a serum PTH value intermediate between the maximal and minimal stimulated PTH values (mid-range value). The value of this "set-point" in subjects with normal parathyroid function is comprised between 1.10 and 1.20 mM.

The calcium "set-point" has been found to be increased in *in vitro* studies using dispersed parathyroid cells from uremic patients, as well as in *in vivo* studies in patients with primary hyperparathyroidism[6]. However, in patients with HPTH-II, there has been conflicting results regarding the calcium "set-point": for several authors it is abnormally increased[7-9], for others it is normal[10-14]. The regulation of the "set-point" by vitamin D therapy is also a controversial issue, we did not find any improvement of the sensitivity of parathyroid cells to calcium after one year of vitamin D treatment[15,16]. However, the differences observed in *in vivo* studies results probably reflect the lack of a standardized methodology. In some studies, total serum cal-

cium concentration was used instead of the biologically more important ionized calcium concentration. In other studies, the variations in serum calcium concentration were obtained by manipulating the calcium concentration in the dialysis bath instead of infusing citrate, EDTA or calcium[13,17,18], and finally, diverse mathematical models have been used to generate the calcium-PTH curve. Nonetheless, most of the *in vivo* findings support the contention that the "set-point" for calcium-regulated PTH secretion is greater than normal in patients with uremic HPTH-II mainly because of a reduced sensitivity of the parathyroid CaR to extracellular calcium[19,20].

The calcimimetics are phenylalkaline compounds that increase the intracellular calcium concentration and inhibit PTH secretion. These drugs act at nanomolar concentrations in bovine parathyroid cells, in *Xenopus laevis* oocytes expressing the bovine or human CaR, and in HEK 293 cells transfected with the CaR. They also potentiate the effect of extracellular calcium on the CaR but do not have any effect in the absence of extracellular calcium. By increasing the sensitivity of the CaR to extracellular calcium they shifted the concentration-response curves for extracellular calcium to the left and reduce the "set-point" for calcium-regulated PTH secretion in *in vitro* studies[5,21].

The steepness of the sigmoid curve becomes also more abrupt indicating an increase in the sensitivity of the CaR to extracellular calcium and a more rapid response of the parathyroid cells to changes in the extracellular calcium concentration. It is therefore plausible that these compounds will also decrease the calcium "set-point" in *in vivo* studies, however, no data has been reported yet, either in animals or in subjects with primary or secondary hyperparathyroidism.

INFORMATION GATHERED FROM GENETICALLY MODIFIED ANIMAL MODELS

Several genetically modified animal models have highlighted the crucial role played by the parathyroid CaR in the control of PTH secretion, parathyroid cell growth, serum calcium and phosphate concentrations, and bone remodeling. The first one is the CaR knock-out mice ($CaR^{-/-}$). Heterozygous mice ($CaR^{+/-}$), similarly to humans with familial hypocalciuric hypercalcemia, have benign and modest elevations of serum calcium and parathyroid hormone levels as well as hypocalciuria. In contrast, homozygous mice ($CaR^{-/-}$), like humans with neonatal severe hyperparathyroidism, have hypercalcemia and markedly elevated serum PTH levels, parathyroid hyperplasia, bone abnormalities, retarded growth, and die shortly after birth because of the effects of severe hypercalcemia. This animal model clearly demonstrates that the absence of CaR is associated with a lack of control of parathyroid cell growth leading to parathyroid hyperplasia[22].

The second animal model was designed to try to dissociate the direct effect of CaR deficiency from the secondary effects of hyperparathyroidism and hypercalcemia on the parathyroid hyperplasia and on the mineral and bone abnormalities. In that model the animals were PTH-deficient ($PTH^{-/-}$) and $CaR^{-/-}$ ($PTH^{-/-}CaR^{-/-}$). This genotype was obtained after inter-crossing mice heterozygous for the null CaR allele with mice heterozygous for a null PTH allele[23]. The genetic ablation of PTH was sufficient to rescue the lethal $CaR^{-/-}$ phenotype. $PTH^{-/-}CaR^{-/-}$ mice survive to adulthood with no obvious difference in size or appearance relative to controls. They exhibited a much wider range of values for serum calcium and renal excretion of calcium than the control littermates, despite the

absence of any circulating PTH, which suggested that the CaR was necessary for the fine regulation of serum calcium levels and renal calcium excretion independent of its effect on PTH secretion. Interestingly, at the parathyroid level, all the $PTH^{-/-}CaR^{-/-}$ mice had enlarged parathyroid glands which emphasized the major role played by the CaR in regulating parathyroid cell growth. It also suggested that parathyroid hyperplasia could occur independently of the altered calcium-mediated control of PTH, because of the absence of CaR, as already shown in the cyclin D1 transgenic mice[24], and that the parathyroid hyperplasia could also occur independently of the overproduction of PTH excluding a paracrine/autocrine role of PTH.

A third model provides more evidences regarding the role of the CaR in the control of PTH secretion and mineral and bone metabolism. The $CaR^{+/-}$ mice were inter-crossed with $Gcm2^{+/-}$ mice to generate $CaR^{-/-}Gcm2^{-/-}$ double mutants. $Gcm2^{-/-}$ mice do not have parathyroid glands because the Gcm2 gene (glial cells missing 2), is a master regulatory gene of parathyroid gland development[25]. In $CaR^{-/-}Gcm2^{-/-}$ mice, the Gcm2 deficiency rescued the perinatal lethality observed in CaR-deficient mice in association with ablation of the parathyroid glands and correction of the severe hyperparathyroidism. In addition, the double homozygous $CaR^{-/-}Gcm2^{-/-}$ mice demonstrated healing of the abnormal bone mineralization observed in the $CaR^{-/-}$ animals, indicating that rickets and osteomalacia in CaR-deficient mice were not due to an independent function of the CaR in bone but to the effect of severe hyperparathyroidism in the neonate. In contrast, concomitant Gcm2 and CaR deficiency failed to rescue the hypocalciuria in CaR-deficient mice, consistent with a direct regulation of urinary calcium excretion through the CaR regulating pathway in the kidney. Interestingly, the $CaR^{-/-}Gcm2^{-/-}$ mice still had measurable serum levels of PTH, which was produced by the thymus. The importance of the role of this thymus source of PTH in humans is uncertain as well as its regulation by serum calcium or calcimimetics drugs, since 4-gland parathyroidectomized patients generally have severe hypoparathyroidism.

The fourth model is represented by mice carrying null alleles of the vitamin D receptor (VDR) gene ($VDR^{-/-}$)[26]. Homozygous mice ($VDR^{-/-}$) are phenotypically normal at birth and live normally at least until 6 months of life. They become hypocalcemic at 21 days of age, at which time their serum PTH levels begin to rise. Hyperparathyroidism is accompanied by an increase in the size of the parathyroid gland as well as an increase in PTH mRNA levels. Rickets and osteomalacia appear by day 35 of age. These mice also develop progressive alopecia from the age of 4 weeks. Interestingly, when $VDR^{-/-}$ mice were fed a diet that prevents secondary hyperparathyroidism in vitamin D-deficient rats this diet normalized growth and serum ionized calcium levels. Moreover, the correction of ionized calcium levels prevented the development of parathyroid hyperplasia and the increase in PTH messenger RNA expression and in serum PTH levels. $VDR^{-/-}$ animals fed this calcium-rich diet did not develop rickets or osteomalacia. This study also demonstrates that normalization of mineral ion homeostasis and the activation of the CaR can prevent the development of hyperparathyroidism, osteomalacia, and rickets in the absence of the genomic actions of 1,25-dihydroxyvitamin D3[27].

Finally, the fifth model comes from the crossing of $VDR^{-/-}$ mice with animals deficient for the gene encoding the $1,25(OH)_2D_3$ synthesizing enzyme the 25 hydroxyvitamin D-1alpha-hydroxylase ($1\alpha(OH)ase^{-/-}$). This double null mutant allowed to examine the effects of calcium and of the $1,25(OH)_2D_3$/VDR system on skeletal

and calcium homeostasis. They showed that an optimal dietary calcium absorption required both $1,25(OH)_2D_3$ and the VDR, that the skeletal mineralization was dependent on adequate ambient calcium but did not directly require the $1,25(OH)_2D_3$/VDR system, and that the PTH secretion was also modulated primarily by ambient serum calcium. However, the enlarged parathyroid glands which those mutants exhibited could only be normalized by the combination of calcium and $1,25(OH)_2D_3$, apparently independently of the VDR. Similarly, optimal osteoclastic bone resorption and osteoblastic bone formation both required an intact $1,25(OH)_2D_3$/VDR machinery. These results indicate that calcium cannot entirely substitute for vitamin D in the control of parathyroid cell growth and in the skeletal and mineral homeostasis but that the two agents have discrete and overlapping functions[28,29].

INFORMATION GATHERED FROM INHERITED HUMAN DISEASES

Inactivating mutations of the CaR gene

The CaR appears to be central to a number of disorders of mineral metabolism. Inactivating mutations in one allele of the receptor lead to a failure by the parathyroid gland to "sense" changes in serum calcium properly and result in an increase in parathyroid hormone secretion. This has been demonstrated to be the molecular basis of familial hypocalciuric hypercalcemia, also known as familial benign hypercalcemia. Generally, these patients are asymptomatic in spite of the constant hypercalcemia which is due to the PTH hypersecretion, the increase in the renal tubular calcium reabsorption, and the increase bone turnover. The hypercalcemia persists after surgical parathyroidectomy, reason for which this act is contraindicated. The parathyroid glands are hyperplasic and appear to have a polyclonal growth pattern[30,31]. This mild and typically asymptomatic disorder is converted into a severe, life-threatening disorder when two inactivated calcium-sensing–receptor alleles are present. Newborns with this condition, called neonatal severe hyperparathyroidism, have severe hypercalcemia, total serum calcium concentration of 15 to 20 mg/dL (3.70 to 5.0 mM), growth retardation, malnutrition, hypotony, constipation, breathing difficulties as well as marked increases in the serum PTH concentration and an important parathyroid polyclonal hyperplasia. They require a surgical parathyroidectomy as fast as possible after birth to be able to survive[32]. These two conditions illustrate again the key role of the CaR in regulation the PTH secretion and parathyroid cell proliferation.

Activating mutations of the CaR gene

Conversely, activating mutations of the CaR have been shown to trick the parathyroid gland into "thinking" that the serum calcium level is elevated when it is not. This false message leads to simultaneous reductions in the serum PTH and calcium levels. These reductions are the cause of autosomal dominant hypoparathyroidism, a disorder that was once classified as a type of idiopathic hypoparathyroidism. Clinically, these patients have a marked hypocalcemia which is often treated by calcium salts and vitamin D derivatives. The hypercalciuria is commonly associated with renal stones and progressive chronic renal failure[33,34]. Patients with this syndrome can be distinguished from patients with idiopathic hypercalciuria because their hypercalciuria is 3 to 4 fold superior[35]. Once more, these observations demonstrate the key role of the CaR in the regulation of PTH secre-

tion. However, no information is available regarding the effect of the activating mutations in the CaR gene on parathyroid cell proliferation and apoptosis rates.

Auto-antibodies specifically directed against the CaR

Since the CaR resides on the cell surface, it was logical to imagine that the CaR could be the target of auto-antibodies and cellular immune attack or of auto-antibodies interfering with the agonist binding sites and activating intracellular signalling pathways. It has now been shown that the CaR may be one of the targets of cellular and humoral immunity in idiopathic autoimmune hypoparathyroidism[36,37]. There are auto-antibodies that inactivate the calcium-sensing receptor and lead to a syndrome very similar to familial hypocalciuric hypercalcemia. In other cases, patients with multiple autoimmune disorders can have specific auto-antibodies direct against the CaR[38] associated with an intermittent, relapsing hypercalcemia and elevated serum PTH levels, which can be responsive to glucocorticoids. When the parathyroid glands of these patients are surgically removed they show an inflammatory involvement. These cases differ from the familial hypocalciuric hypercalcemia because the serum calcium level can be entirely normal, and because the elevations in the serum calcium and PTH levels responded to glucocorticoids.

It has also been learned that auto-antibodies may functionally activate the CaR in idiopathic hypoparathyroidism in a scenario parallel to the autoimmune activation of thyrotropin receptors in Graves' disease[39]. Generally, these auto-antibodies are of the IgG type 4 which do not involve the activation of the complement cascade and do not result in the destruction of parathyroid cells. They only interfere with CaR binding sites and modulate its activity. The use of these kinds of antibodies could be another way to target the parathyroid CaR and to treat either hypofunctionning or hyperfunctionning parathyroid states.

PARATHYROID HYPERPLASIA PREVENTION AND REGRESSION IN UREMIC ANIMAL MODELS OF SECONDARY HYPERPARATHYROIDISM

There is a great hope regarding the issue whether the calcimimetics through the activation of the parathyroid CaR could prevent, stop or reduce the parathyroid hyperplasia seen in HPTH-II. The first studies in 1997 with NPS R-568, a first generation calcimimetic, showed that in 5/6 nephrectomized rats, the infusion of NPS R-568 intraperitoneally simultaneously with 5-bromodeoxyuridine (BrdU) to label S-phase cells, reduced the number of BrdU-positive PT cells of vehicle-treated nephrectomized by 50% compared to that of sham-operated animals[40].

In the second study, the CRF was created by ligation of the renal arteries and the severe secondary HPT induced by a high-phosphorus diet. The high-phosphorus diet was started 6 days after the surgery, and NPS R-568 was administered to the rats for 56 days either by daily gavage (30 or 100 micromol/kg) or by continuous subcutaneous infusion (20 micromol/kg./day). Serum PTH levels were maintained at levels comparable with those of sham-operated controls. Parathyroid gland enlargement was caused predominantly by hyperplasia and the total parathyroid cell number per kg of body weight was 3.5-fold higher in vehicle-treated CRF rats than in sham-operated controls. Both infusion and high-dose gavage of NPS R-568 completely prevented the increase in parathyroid cell number [41].

The third study was designed to examined the effects of daily oral gavage or continuous

subcutaneous infusion for 8 wk of NPS R-568 on the progression of already established mild or moderate-to-severe HPTH-II in rats with CRF induced by 5/6 nephrectomy. Both oral and infused NPS R-568 completely prevented further hyperplasia but did not reduce total parathyroid cell number below that present at the initiation of treatment.

Finally, the last published study examined the effect of the second generation calcimimetic cinalcalcet HCl, given by gavage daily at the doses of 1, 5, and 10 mg/kg, for 4 weeks, starting 6 weeks after the creation of CRF by 5/6 nephrectomy, on parathyroid cell proliferation and apoptosis. The results showed that cinalcalcet at the dose of 5 and 10 mg/kg significantly reduced the number of proliferating cells and decreased parathyroid gland weight compared with vehicle-treated 5/6 nephrectomized rats. There was no difference in the apoptosis rate from cinacalcet HCl-treated or vehicle-treated rats[42].

The effects of these calcimimetics on preventing and attenuating parathyroid cell hyperplasia in CRF animals were independent of changes in serum $1,25OH_2D_3$, total calcium or phosphorus, suggesting a dominant role for the calcium receptor in regulating parathyroid cell proliferation.

REGULATION OF THE PARATHYROID CAR, UP-REGULATION BY ITS OWN AGONISTS

The CaR mRNA expression in parathyroid cells is down-regulated in CRF, which contributes to the reduced response of these cells to the serum calcium concentration and to the stimulation of PTH synthesis. This down-regulation is probably the result of a combination of factors, namely low serum calcium and vitamin D[43-45], high phosphorus and uremic toxins[46] (Table I). The parathyroid CaR is also up-regulated by the age probably in response to the usual decrease in the calcium and vitamin D balance observed with ageing[47]. Interleukin 1 (IL-1) is well known as a potent inhibitor of PTH secretion, it is now established that its negative effect on PTH secretion involves the up-regulation of the CaR[48]. Interestingly, the parathyroid CaR can be also pharmacologically up-regulated by the administration of calcimimetics[49]. This phenomenon may explain why patients with severe HPTH-II, with certainly nodular parathyroid tumors and reduced CaR, still respond satisfactorily to the calcimimetics treatment[49].

CORRECTION OF HPTH-II BY CALCIMIMETICS IN UREMIC PATIENTS

There are now convincing evidences that the calcimimetics can efficiently control at short-term (26 weeks) and at long-term (> 3 years) and probably stop the progression of HPTH-II in ESRD patients treated by dialysis[50] (Table 1). Cinacalcet HCl (Sensipar®), which has been the most extensively studied second generation calcimimetic, has been approved in the United States by the Food and Drug Administration, and now in the European Community, for the treat-

Table I – Regulation of the CaR mRNA expression in parathyroid cells

Age	Increase
Calcimimetics	Increase
Calcium	Increase
Interleukin 1	Increase
Phosphorus	Decrease
Uremic toxins	Decrease
Vitamin D	Increase

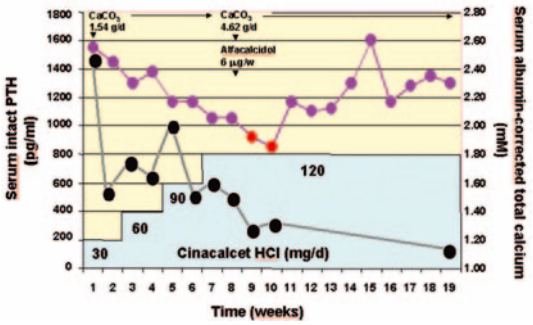

Figure legend. This graph shows the dose-depend reduction in serum PTH and total calcium in a hemodialysis patient treated by cinacalcet HCl for a severe secondary hyperparathyroidism. Abbreviations are : CaCO3, calcium carbonate; PTH, parathryroid hormone.

Figure 1. Treatment of renal secondary hyperparathyroidism with cinacalcet HCl

ment of HPTH-II (serum PTH level > 300 pg/ml) in dialysis patients and hypercalcemia in patients with parathyroid carcinoma.

In dialysis patients with HPTH-II the optimal daily dose of cinacalcet HCl appears to be between 60-120 mg, however, severe cases may require the maximal daily doses of 180 mg. Independently of the baseline PTH value, PTH decreases by 60-70% 2 to 4 hours after its administration and remains low for the next 24 hours. Serum total calcium follows the same trend that PTH but delayed of 2-4 hours, it often decreases by 20-30% of its baseline value. The reduction in serum PTH, seen during the first weeks of treatment, can attain up to 90% of the baseline value, similar to a surgical PTX (Figure 1). A transient hypocalcemia often occurs during this phase, which is in part due to the phenomenon of "hungry bone"[51], and also to a decrease in the intestinal expression of the TRPV-5 calcium transporter[52]. Its correction needs an increase in the dietary calcium intake or high doses of calcium salts alone or in combination with vitamin D (Figure 1).

Table II illustrates the main results obtained with cinacalcet HCl in phase III studies in dialysis patients with HPTH-II. Cinacalcet HCl, at the doses of 20-180 mg/d, reduced mean serum PTH levels of 33% and 65% after 18 weeks and 3 years of treatment respectively[50,53-57]. Mean serum calcium x phosphorus product showed a decrease of 6 to 15%. This treatment allows

Table II – Principal results of phase III studies with cinacalcet HCl in dialysis patients with secondary hyperparathyroidism

Reference	Number of patients	Duration of the study	Dose of cinacalcet HCl	PTH Results (% decrease)	Ca x P (% decrease)
Block et al. (50)	371	26 weeks	30-180 mg/d	- 43%	- 15%
Mittman et al. (56)*	34	6 weeks	30-180 mg/d	- 50%	- 13%
Quarles et al. (54)	36	52 weeks	30-100 mg/d	- 33%	- 7.9%
Lindberg et al. (55)	29	18 weeks	20-50 mg/d	- 26%	- 11%
Urena et al. (53)	6	3 years	30-180 mg/d	- 65%	- 6%
Bushinsky et al (57)	444	16 weeks	30-180 mg/d	- 62%	?

Table III – Cinacalcet HCl treatment allows to achieve more frquently the National Kidney Foundation-Kidney Disease Outcomes Quality Initiative (NKF-K/DOQI) targets

NKF-K/DOQI Targets	Placebo (%)	Cinacalcet HCl (%)
iPTH ≤ 300 pg/ml	10	56
Ca x P ≤ 55 mg^2/dl^2	33	46
Phosphorus ≤ 5.5 mg/dl	37	54
Calcium 8.4-9.5 mg/dl	24	49
iPTH and CaxP targets	6	41

Reference : (58)

to achieve more frequently the National Kidney Foundation-Kidney Disease Outcomes Quality Initiative (NKF-K/DOQI) recommended targets values for serum calcium, phosphorus, PTH and Ca x P product[58] (Table III).

Altogether, the results of these clinical studies reinforce the hypothesis that targeting and activating the parathyroid CaR by the calcimimetics is a highly and specific way to efficiently treat HPTH-II in ESRD patients.

CONCLUSIONS

Targeting the CaR with the calcimimetics is actually the only way to specifically decrease PTH secretion without increasing serum calcium phosphorus product. The effectiveness of these drugs on the control of PTH secretion, and the simultaneous reduction in serum calcium-phosphorus product, make them advantageous over the classical therapies of HPTH-II. However, long-term studies will be needed to evaluate its potential beneficial effects on mortality rate, skeletal fracture incidence, the prevention of cardiovascular morbidity. These studies could also provide us with an evaluation of the cost-effectiveness of calcimimetics versus traditional treatments of HPTH-II.

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