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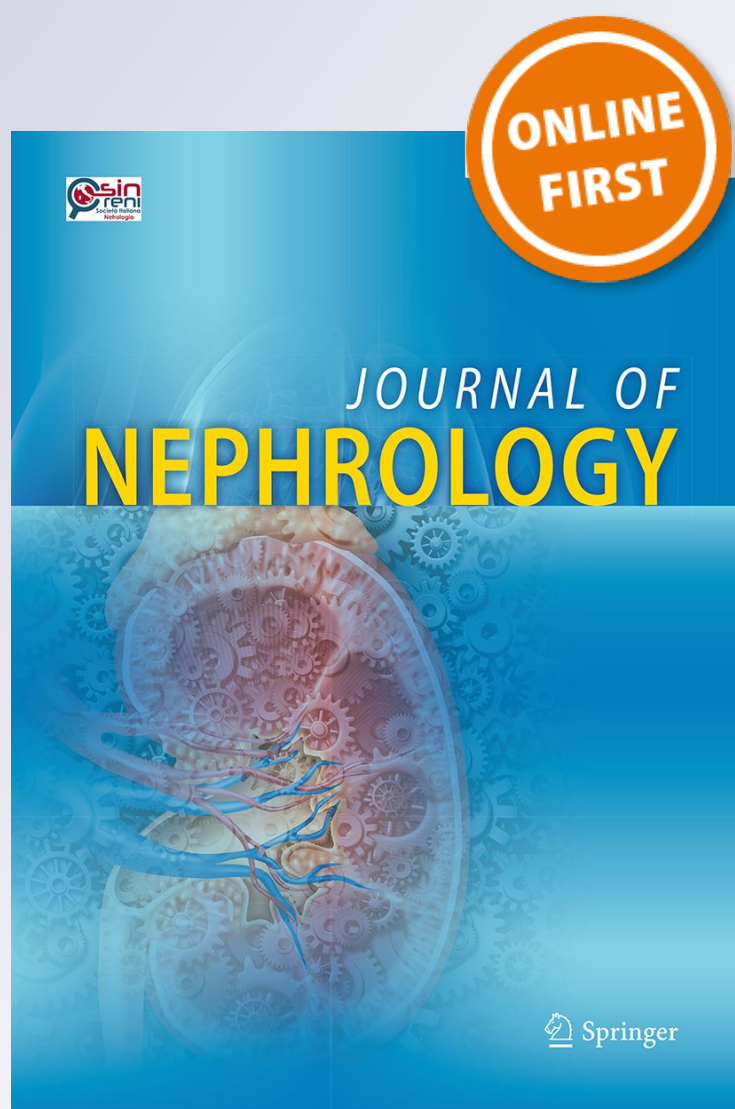
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Journal of Nephrology

ISSN 1121-8428

J Nephrol

DOI 10.1007/s40620-017-0410-1



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Positioning novel biologicals in CKD-mineral and bone disorders

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Received: 2 March 2017 / Accepted: 15 May 2017
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Abstract Renal osteodystrophy (ROD), the histologic bone lesions of chronic kidney disease (CKD), is now included in a wider syndrome with laboratory abnormalities of mineral metabolism and extra-skeletal calcifications or CKD-mineral and bone disorders (CKD-MBD), to highlight the increased burden of mortality. Aging people, frequently identified as early CKD, could suffer from either the classical age-related osteoporosis (OP) or ROD. Distinguishing between these two bone diseases may not be easy without bone biopsy. In any case, besides classical therapies for ROD, nephrologists are now challenged by the possibility of using new drugs developed for OP. Importantly, while therapies for ROD mostly aim at controlling parathyroid secretion with bone effects regarded as indirect, new drugs for OP directly modulate bone cells activity. Thus, their action could be useful in specific types of ROD. Parathyroid hormone therapy, which is anabolic in OP, could be useful in renal patients with low turnover bone disease. Denosumab, the monoclonal antibody against receptor activator of NF- κ B ligand (RANK-L) that inhibits osteoclast activity and proliferation, could be beneficial in cases with high turnover bone. Use of romosozumab, the monoclonal antibody against sclerostin, which both stimulates osteoblasts and inhibits osteoclasts, could allow both anabolic

and anti-resorptive effects. However, we should not forget the systemic role now attributed to CKD-MBD. In fact, therapies targeting bone cells activity could also result in unpredicted extra-bone effects and affect cardiovascular outcomes. In conclusion, the new biologicals established for OP could be useful in renal patients with either OP or ROD. In addition, their potential non-bone effects warrant investigation.

Keywords Renal osteodystrophy · Osteoporosis · Mineral bone disorders · Bone mineral density · PTH 1–34 · Teriparatide · Denosumab · Romosozumab.

Introduction

The complex derangements in biomarkers of mineral metabolism occurring in chronic kidney disease (CKD) are invariably associated with bone abnormalities and extra-skeletal calcification. These three aspects (laboratory, bone and vessels) are metabolically related and have been combined in a new clinical condition named “CKD-mineral bone disorders” (CKD-MBD) [1]. The clinical spectrum of CKD-MBD goes beyond bone lesions to include an increased burden of morbidity and mortality for any cause or for cardiovascular disease. Thus the term renal osteodystrophy (ROD) now indicates a single component of this “syndrome” [2], namely the bone pathology associated with CKD-MBD that develops as a consequence of chronic renal failure (CRF) [3]. Available histologic data of ROD are scarce and mostly limited to the overt, late stages of renal failure. Classical categories of ROD are high-turnover bone, low-turnover bone, osteomalacia and mixed uremic bone lesions. However, more recently a new classification of ROD has been proposed to take into account the three

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bone histologic parameters that are most relevant for bone mechanical and metabolic performances: turnover, mineralization and volume (the so-called “TMV” classification) [4]. Bone biopsy is the gold standard for determining the different types of ROD since the available biochemical markers [5] are not completely reliable.

Further, in recent years there has been increased attention to the early phases of CKD that are so commonly evidenced in elderly people. Some reduction in glomerular filtration rate (GFR) invariably occurs with aging as a result of the physiologic reduction in the number of nephrons; however, the aging-related vascular disease or other nephropathies typical of aging may add further damage and lead to a further reduction of GFR. Since aging people also suffer osteoporosis (OP), we need to appreciate its coexistence and interrelation with ROD. OP is defined as a condition of reduced bone strength predisposing to an increased risk of fracture [6], and is mostly diagnosed in the adult and aging population by the instrumental evidence of reduced bone mineral density (BMD) assessed by dual energy X-ray absorptiometry (DXA). Histologically, OP is characterized by a reduction of bone volume, but differs from ROD in that the defective mineralization processes are absent. For this reason, assessment of BMD in renal patients has been considered inadequate and discouraged until recently [3]. Accordingly, renal patients have been always diagnosed as affected with any type of ROD but not with OP. In fact, there is not still agreement on how to recognize OP in CKD [7]. Nonetheless, recent epidemiologic data demonstrated that CKD patients with reduced BMD suffer an increased risk of fracture and the eventual clinical burden of morbidity and mortality [8]. Further, the wider clinical spectrum of CKD-MBD including not only ROD with its burden of fracture risk, but also the increased cardiovascular mortality, suggests that, in the early phases of CKD, we should search not only for mineral metabolism derangements and ROD but also for possible OP [9]. Indeed, the earliest metabolic modification of mineral metabolism detectable in CKD seems to be a reduction in circulating soluble Klotho, which is a protein synthesized by renal tubular cells [10]. Klotho is the co-receptor of FGF23, the recently appreciated bone hormone involved in the regulation of phosphate, vitamin D and parathyroid hormone, whose circulating levels increase along with Klotho reduction [10]. These early alterations in Klotho and FGF23 are regarded as the very early disturbance of the bone-kidney cross-talk [11] which represents one of the pathophysiologic components of CKD-MBD. Accordingly, early changes in Klotho/FGF23 could be helpful to distinguish patients with CKD and initial ROD from non-CKD pure OP patients. In addition, we could raise the question if there is a GFR threshold level for the switch from OP to ROD. Indeed, no reliable answer is available. We can speculate that, since FGF23 reflects

bone cells activity, its early increments could be indicative of the adaptation of bone to CKD, and thus of the dawn of ROD. Accordingly, but speculatively, a threshold estimated GFR (eGFR) could be set at 60 ml/min. Indeed, a recent paper in OP men evidenced that higher FGF23 quartiles are useful predictors of incident fractures only in cases with $eGFR_{CrCys} < 60$ ml/min (a recognized GFR threshold for increments of FGF23) and not in the population as a whole [12].

These premises are necessary to highlight that ROD classification, either classical or TMV, is currently appreciated mostly in advanced renal disease while some room should be found for OP, in particular in the early phases of CKD. Also, the epidemiologic evidence that a significant reduction in BMD (diagnostic for OP) identifies CKD patients at increased risk of fracture [8, 13], and underlines the need for early therapy. Indeed, new therapies with drugs developed specifically for OP could be employed in CKD. Standard therapies for ROD mainly aim at improving the biochemical control of secondary hyperparathyroidism with the positive bone effects regarded as mostly indirect. At variance, new drugs developed to treat OP can directly modulate the activity of osteoblasts and osteoclasts and could thus represent new options to employ in CKD patients with either OP or ROD. Three drugs, in particular, merit attention from nephrologists: parathyroid hormone (PTH), denosumab and romosozumab. All of them have been successfully employed to increase BMD and reduce fracture rates in OP patients. The aim of this review is to examine the pharmacologic characteristics and clinical performance of these three drugs in order to allow an appraisal of their possible use in CKD. For this reason, we will first recapitulate the pathophysiologic differences of bone in CKD and OP and will then consider, for each drug, the standard therapeutic indications and their potential role in CKD.

Bone physiology and alterations in CKD and osteoporosis

Bone is an endocrine organ whose cells constantly renew. In physiologic condition, there is a coordinated and persistent process of bone resorption (worn bone) and bone formation (new bone) which needs to be well balanced (Fig. 1). Osteoclasts are responsible for resorption and osteoblasts for formation. Both processes are strictly regulated by hormones with paracrine or endocrine actions. Osteoclasts are members of the monocyte/macrophage family and bone marrow macrophages are their principal precursors. Two cytokines are indispensable for osteoclastogenesis and osteoclast activation: receptor activator of NF- κ B ligand (RANK-L) [14] and macrophage colony-stimulating

factor 1 (M-CSF) [15]. Osteoclast precursors express both RANK-L and M-CSF receptors (RANK and M-CSF-R, respectively) and, in the presence of M-CSF, RANK-L promotes the differentiation of monocytes into mature bone-resorbing osteoclasts. The major source of RANK-L and M-CSF are bone marrow stromal cells and their derivative osteoblasts [14]. Osteoblasts are also the main source of osteoprotegerin (OPG) production. OPG, a high-affinity ligand of RANK-L, acts as soluble inhibitor of RANK-L thus reducing osteoclast differentiation [16]. Finally, also osteocytes, the terminally differentiated osteoblast, are involved in regulation of bone turnover. In fact, they secrete both RANK-L and sclerostin. Sclerostin, an anti-anabolic protein, exerts its action by inhibiting the canonical Wnt (Wingless type mouse mammary tumor virus integration site) pathway. Sclerostin reduces osteoblastogenesis and promotes osteoblast and osteocyte apoptosis [17]. Moreover, sclerostin induces RANK-L synthesis and stimulates osteoclastogenesis. Both the RANK/RANK-L/OPG axis and Wnt-sclerostin pathway are now recognized as essential for the fine-tuned regulation of bone remodeling that needs to be well balanced (i.e. bone formation should equal bone resorption). Any uncoupling between resorption and formation leads to disease. In OP, for example, bone resorption prevails over bone formation thus leading to a progressive reduction of bone mass and reduced bone strength. In ROD, the underlying metabolic derangements are more complex and involve bone cells activity and proliferation and the systemic balance in calcium and phosphate. Two hormones are classically involved with ROD development: parathyroid hormone and vitamin D. Parathyroid hormone secretion is typically increased in chronic renal failure (CRF) and produces a direct stimulation of bone cells, by activating its receptor, namely PTH receptor 1, on osteoblasts. This increases osteoblast maturation and activity, including an increased synthesis of the above-mentioned

RANK-L, with eventual osteoclast activation. Also for vitamin D, whose synthesis is mostly reduced in CRF, there is evidence of the influence of a specific receptor, the vitamin D receptor (VDR), on bone cells. Through this receptor, vitamin D directly modifies bone cells activity, independently of other renal and intestinal actions affecting divalent ions metabolism. In bone, vitamin D stimulates calcium resorption [18] and osteoclast differentiation through induction of RANK-L synthesis [19]. Accordingly, the different types of ROD will develop depending on the prevailing hormonal derangement. Table 1, which recapitulates

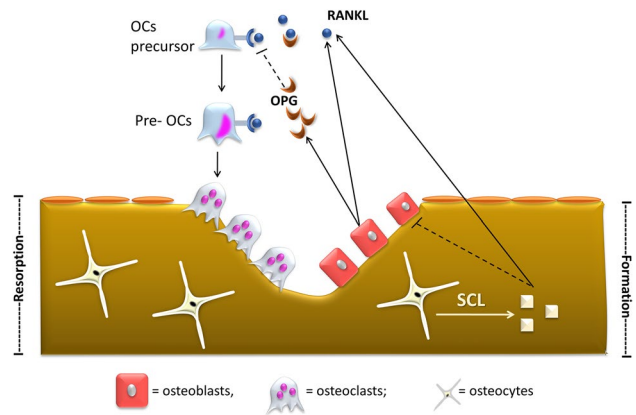


Fig. 1 Bone Remodeling. In physiologic condition, bone is continuously renewed through the coordinated process of bone resorption by osteoclasts and bone formation by osteoblasts. Receptor activator of NF- κ B ligand (RANKL) and osteoprotegerin (OPG), both produced by osteoblasts, are the key regulators of bone remodeling. RANK-L promotes the differentiation of osteoclasts' precursor (OCs precursor) and pre-osteoclasts (Pre-OCs) in mature osteoclasts. OPG, a high-affinity ligand of RANK-L, acts as a soluble inhibitor of RANK-L thus reducing osteoclasts differentiation. Osteocytes regulate bone turnover by secreting sclerostin (Scl). Sclerostin reduces osteoblastogenesis, promotes osteoblast and osteocyte apoptosis and, by inducing RANK-L synthesis, stimulates osteoclastogenesis

Table 1 Derangements in bone turnover, mineralization and volume in ROD and in OP

Classical definition	T	M	V	Definition
Renal osteodystrophy				
Osteitis fibrosa	High	Abnormal	High/normal/low	Increased bone remodeling with peritrabecular fibrosis. Increased number and activity of osteoclasts and osteoblasts
Osteomalacia	Low	Abnormal	Normal/low	Defective mineralization of the organic matrix of bone
Adynamic bone disease	Low	Normal/abnormal	Normal/low	Low bone formation, low number of bone cells
Mixed uremic osteodystrophy	High- normal	Abnormal	Normal/low	Features of both osteitis fibrosa and osteomalacia
Osteoporosis				
High turnover OP	High	Normal	Low	Uncoupling of bone cells activity with prevailing resorption
Normal/low turnover OP	Low	Normal	Low	Uncoupling of bone cells activity with prevailing low bone apposition

ROD renal osteodystrophy, OP osteoporosis, T turnover, M mineralization, V volume

the main alterations in bone turnover, mineralization and volume associated with OP and ROD, summarizes the differences and similarities existing between these two bone diseases.

Parathyroid hormone

Two biological PTH molecules are available for clinical use. One, the full-length recombinant human PTH (rhPTH 1–84) is commercialized in the United States for patients with hypoparathyroidism and therefore we will not consider it further. The other one, the N-term recombinant human PTH (rhPTH 1–34 or teriparatide), is produced by *Escherichia Coli* with recombinant DNA technology, consists of the first 34 amino acids of endogenous PTH and has been approved since 2003 for the treatment of OP. Pharmacokinetics, contraindications, collateral effects, dosing and duration of therapy are schematically illustrated in Table 2. The presence in this molecule of the active N-term region of PTH suggests that all the biological actions of natural PTH are predictable. Therefore, to appreciate the potential of this drug, it may be useful to recall some essential information about PTH, an 84-amino acid peptide secreted by

parathyroid glands in response to the shifts in serum calcium. Any decrease in serum ionized calcium stimulates PTH secretion, which in turn increases calcium reabsorption in the renal tubule and in bone [20]. Further, in renal tubular cells PTH stimulates the conversion of 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D thus increasing intestinal calcium absorption [21]. Both actions aim at restoring serum calcium levels and at inhibiting, as a negative feed-back, PTH secretion. Natural circulating PTH comprises the intact molecule (PTH 1–84) and several amino (N)- and carboxy (C)-terminal fragments. The intact molecule and the N-terminal fragments (e.g. PTH 1–34), are active calcemic peptides since they bind to a specific PTH receptor, known as PTH1R [22], which is selective for the N-terminal region of the molecule [23] and expressed in the PTH target cells. At variance, the C-terminal fragments do not bind to this receptor and have been since considered inactive. More recently, experimental evidence suggested that a paradoxical hypocalcemic activity could be exerted by the C-terminal fragments through a specific but still unidentified C-term PTH receptor (C-PTHRs) [24]. Another detail useful to appreciate the bone effects of PTH is that the PTH1R is expressed in osteoblasts but not in osteoclasts and that its activation increases osteoblasts'

Table 2 Comparison of the main pharmacologic and clinical differences among the three biological drugs examined

	Teriparatide	Denosumab	Romosozumab
MW	4.1 kDa	35.4 kDa	145.9 kDa
Half life	eGFR ≥ 60 ml/min/1.73 m ² : 1.2 h eGFR 30–59.9 ml/min/1.73 m ² : 1.5 h eGFR 15–29.9 ml/min/1.73 m ² : 5 h	Normal renal function and CKD (stages 1–5D): 25.4 days	Normal GFR: 6–9 days Reduced GFR: No data available
Dosing	Intermittent: 20 or 40 mcg/daily Weekly: 56.5 mcg	OP: 60 mg every 6 months Hypercalcemia or malignancy: 120 mg every 4 months	OP: 70–140 or 210 mg monthly or 140–210 every 3 months
Route of administration	Subcutaneous	Subcutaneous	Subcutaneous
Duration of therapy	Max 24 months	24 months	12 months
Side effects	<i>Short term</i> hypercalcemia, hypercalciuria, hyperuricemia, hypotension <i>Long term</i> Osteosarcoma (in rats)	Skin reactions Hypocalcemia	Skin reactions
Indications	Severe OP OP in patients intolerant or with contraindication to bisphosphonates OP in patients unresponsive to other therapies	OP and high risk of fractures OP in patients intolerant or with contraindication, or unresponsive, to other therapies Hypercalcemia of malignancy refractory to bisphosphonates Tumors: prevention of skeletal related events in patients with metastases	Post-menopausal OP (Phase III clinical studies)
Contraindications	Hypercalcemic disorders Predisposition to osteosarcoma Primary or secondary hyperparathyroidism Nephrolithiasis	Hypocalcemia Vitamin D deficiency (replace with vitamin D prior to administration)	Not available
Bone effect	Anabolic	Anti-resorptive	Anabolic and anti-resorptive

MW molecular weight, eGFR estimated glomerular filtration rate, OP osteoporosis, CKD chronic kidney disease, BMD bone mineral density

number and activity. Accordingly, an anabolic action is invariably attributed to PTH [25] (Fig. 2). However, following activation, osteoblasts also increase RANK-L synthesis [26], which in turn will recruit new osteoclasts and activate them, thus balancing the two processes of bone formation and resorption. The final bone balance could nonetheless be affected by another characteristic of PTH hormone: its secretion is rhythmic and, with mechanisms still to be discovered, intermittent secretion is anabolic while chronic stable secretion is catabolic. Therefore, considering the short half-life of PTH, its daily administration as a drug resembles a pulsatile secretion and, not surprisingly, resulted in anabolic effects with increments in BMD in both preclinical [27, 28] and clinical studies [27, 28]. Also biochemical markers of bone formation like bone alkaline phosphatase and procollagen type I C-terminal propeptide (PICP) have been reported to increase [29]. Conceivably, since osteoblast stimulation by PTH eventually leads to osteoclast activation, as confirmed by the late increments of biomarkers of bone resorption [23], the initial increase in BMD could be caught up with by resorption in the long term. As a matter of fact, the stimulation of bone formation occurs earlier than bone resorption. Therefore, the rate of BMD increment is predictably higher in the first months of therapy and expected to plateau as also osteoclasts become recruited [23, 25, 29]. The final balance of BMD will reset at a value a little bit higher. On the contrary, chronic increments of PTH, like in primary hyperparathyroidism, may

result in bone catabolism. Accordingly, the issue of the drug's half-life may be essential when considering teriparatide use in renal patients.

Despite a powerful anabolic action when administered once daily, PTH 1–34 is not a first-line therapy for OP due to its high costs, impractical route of administration and the consolidated experience with other common therapies. Teriparatide use is indicated for patients with severe primary or secondary OP (like glucocorticoid-induced OP) and high risk of fracture who are intolerant or unresponsive to standard therapies. Notably, the effect of PTH is dose dependent [29] and site specific. Trabecular bone increases more than cortical bone, as evidenced by the greater increase of BMD in trabecular sites (like vertebrae) compared to cortical regions (like radius) [30, 31]. PTH effects are not persistent since the BMD increment is lost after discontinuation of therapy, unless antiresorptive agents are administered [32]. Pharmacokinetic studies in volunteers with CKD show an increased half-life along with GFR reduction, with the standard half-life of 1.2 h in normal subjects increasing up to 5 h in patients with GFR 15–30 ml/min/1.73 m² [33]. In any case, all teriparatide is eliminated within 24 h. Safety and efficacy of teriparatide have been evaluated in a therapeutic trial involving 1637 postmenopausal women with OP, aged 42–86 years, and whose serum creatinine at enrollment was ≤ 2 mg/dl. A further inclusion criterion was no increment in serum or urine calcium, or of PTH [34]. Patients were randomized to receive teriparatide (20 mcg or 40 mcg) or placebo and were arbitrarily divided into three groups of GFR: normal (GFR ≥ 80 ml/min/1.73 m²), mildly reduced GFR (50–79 ml/min/1.73 m²) and moderately reduced (GFR 30–49 ml/min/1.73 m²). Patients with mild or moderate renal impairment were older and with lower baseline BMD (both neck and femoral). However, compared to placebo, teriparatide (either dose) increased lumbar spine BMD in each renal function subgroup, and femoral neck BMD only in subgroups of normal and mildly reduced GFR. Fracture risk, both vertebral and non-vertebral, was similarly reduced in patients with normal or reduced GFR. Treatment with teriparatide increased circulating biomarkers of bone formation (amino-terminal peptide of procollagen type I, PINP), while markers of bone resorption were not analyzed in this study. Notably, patients with renal impairment experienced an increased incidence of hyperuricemia and, importantly, of hypercalcemia which, however, was always < 11 mg/dl with the lower dose of 20 mcg. Notably, this study enrolled patients with BMD values diagnostic of OP and without overt hyperparathyroidism. However, since biochemical evidence of SHP may be subtle and difficult to recognize in the early phases of renal disease, the presence of ROD

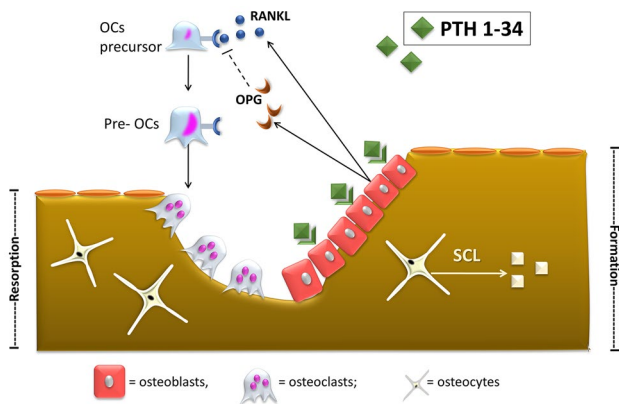


Fig. 2 Effects of PTH 1–34 on bone. Intermittent administration of recombinant human PTH 1–34 stimulates bone formation. The positive effect of intermittent PTH on bone appears to be mediated through the PTH-1 receptor (PTH1R), expressed only in osteoblasts. PTH1R activation increases osteoblasts' number and activity. However, osteoblasts activation by PTH increases receptor activator of NF- κ B ligand (RANK-L) synthesis and decreases osteoprotegerin (OPG) synthesis. This, in turn, leads to new osteoclasts recruitment and activation, thus balancing the two processes of bone formation and resorption. However, the increment in bone formation occurs earlier than that of bone resorption, thus increasing bone volume in the first months of therapy before osteoclast activation

instead of OP cannot be ruled out. Indeed, recent evidence suggests that adynamic bone is the first manifestation of ROD, secondary to bone resistance to PTH in the early phases of renal failure [35, 36] and this should not be a contraindication to PTH therapy.

As for hemodialysis (HD) patients, the clinical experience with teriparatide is very limited. One study enrolled patients with a low concentration of PTH, low bone mass defined as low BMD at femoral, lumbar or distal one-third radio (T score ≤ -2.5), associated with a history of fragility fractures [37]. Teriparatide was administered once-weekly (56.5 mcg) instead of the standard daily schedule and associated with increased lumbar spine BMD starting from week 24 and no changes in femoral BMD, thus confirming the site-specific effects. Markers of bone formation, such as PINP and bone alkaline phosphatase (AP), increased early, while markers of bone resorption (tartrate-resistant acid phosphatase 5, TRAP5b) gradually decreased. Serum PTH levels increased at week 4 and remained higher up to week 24. Notably, and at variance with data in non-dialysis renal patients, a reduction in serum calcium was also observed, even though in the absence of recorded symptoms.

In summary, in early to moderate CKD, teriparatide could reduce fracture risk and increase BMD of trabecular bone, in particular. This effect is most probable in postmenopausal women with mildly reduced GFR, low BMD and no biochemical evidence of SHP. In HD patients with low PTH and hypothetical adynamic bone, teriparatide could increase lumbar BMD. In theory, PTH therapy could be beneficial in the presence of low bone volume and turnover without osteomalacia, while it should be avoided in other types of ROD. Accordingly, we could try teriparatide mainly in patients with adynamic bone and low BMD. In any case, the complexity of bone metabolism in renal failure does not rule out *a priori* the use of this drug in renal patients even with moderately increased PTH levels that do not exclude adynamic bone disease. Further, evaluation of systemic effects is warranted in renal patients, since PTH is not only a bone hormone, but also a uremic toxin whose levels are associated with mortality [38], and it is now acknowledged to be a stimulator of FGF23 [39] which, in turn, is a recognized inducer of left ventricular hypertrophy in uremia [40]. Absence of renal function carries with it potential problems which merit consideration. Since PTH pulsatile secretion is anabolic while chronic and stable increments are catabolic for bone, we can speculate that absence of renal function by prolonging the half-life of the drug could modify its efficacy. Further, drug metabolites with unpredicted actions could be produced, again affecting the final clinical result. Randomized clinical trials are warranted.

Denosumab

Denosumab is a human monoclonal IgG2 antibody, produced in mammalian cell lines by recombinant DNA technology, which targets and binds RANK-L with high affinity and specificity. RANK-L is a cytokine, member of the TNF superfamily, essential for osteoclastogenesis and osteoclast activation. RANKL also has a function in the immune system, since it is expressed by T helper cells and is thought to be involved in dendritic cell maturation. RANK-L knockout mice develop severe osteopetrosis and low osteoclasts number but also defective T and B lymphocytes differentiation [41]. Experimental inhibition of RANK-L with antibodies reduces osteoclast activity and survival with eventual reduction of bone resorption sites and increased BMD (Fig. 3). In animal models of OP [42] or of glucocorticoid-induced OP [43], RANK-L inhibition reduced bone loss and increased BMD. Based on this experimental evidence, a fully human monoclonal antibody to the RANK-L has been made available since 2010 for OP and glucocorticoid-induced OP treatment. In postmenopausal women, denosumab significantly increased BMD by 9.2% at the lumbar spine and 6.0% at the total hip [44] and the increment was greater than with alendronate [45]. In addition to improving BMD, denosumab reduced fracture risk, at vertebral and non-vertebral sites [44]. To date, denosumab is not indicated as first-line osteoporosis treatment and its use is limited to patients intolerant or unresponsive to other standard therapies (such as bisphosphonates). In patients at high risk for fracture who also have impaired renal function, denosumab could be useful since, at variance with bisphosphonates, no dosage adjustment is required. In fact,

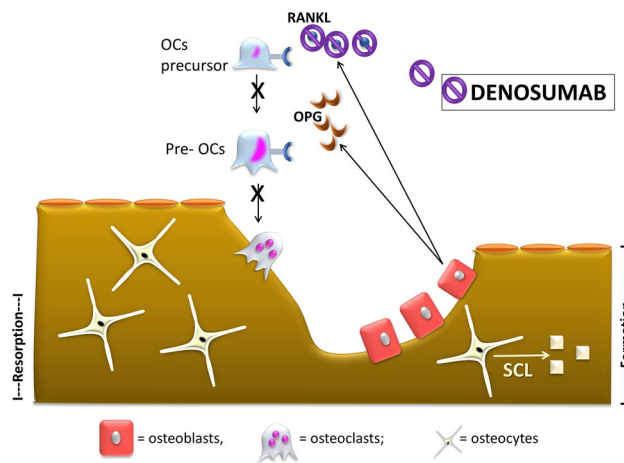


Fig. 3 Effects of denosumab on bone. Denosumab targets and binds receptor activator of NF- κ B ligand (RANK-L) with high affinity and specificity. RANK-L inhibition leads to suppression of osteoclasts differentiation, activity and survival. The final effect is the reduction of bone resorption sites and increased bone volume

a pharmacokinetic study in patients with different degrees of renal insufficiency showed no difference in serum concentration of denosumab along with reduction of GFR [46]. However, in renal patients episodes of hypocalcemia were more severe and prolonged than in subjects with normal renal function and were more likely in those with worse renal function [46]. This is consistent with a significant inhibition of osteoclast activity which, in renal patients with high PTH levels, is expected to lower not only serum calcium but also serum phosphate, similarly to parathyroidectomy. The safety and efficacy of denosumab in patients with renal impairment have been evaluated in a post-hoc analysis of the Fracture Reduction Evaluation of Denosumab in Osteoporosis Every 6 Months (FREEDOM) trial [44] involving 7808 postmenopausal women, 3902 treated with denosumab and 3906 with placebo. In the secondary data analysis, GFR was estimated with the Cockcroft and Gault formula with the aim of evaluating if, in the different classes of GFR, there was a different efficacy [47]. Interestingly, increments in BMD and incidence of adverse events (including changes in creatinine and calcium) did not differ by CKD stages. Anyway, in this analysis the distribution of cases within the different classes of GFR was not balanced (there was no patient in stage 5, 73 in stage 4, 2817 in stage 3, 4069 in stage in 2 and 842 in stage 1) and the presence of kidney disease was not verified (no data provided on proteinuria or urinary or kidney abnormalities). In addition, values of PTH were not reported nor was the type of bone disease verified (OP or ROD?). Accordingly, the conclusions on safety and efficacy should be limited to postmenopausal women with GFR > 30 ml/min and without overt renal disease. In dialysis patients, the employment of denosumab is very limited mostly due to the risk of severe and prolonged hypocalcemia. In a pilot study involving 12 HD patients diagnosed to have OP - based however, on calcaneal ultrasound (US) T-Score values and not on standard DXA - the effect of denosumab was evaluated on US-BMD and fracture risk. Changes in both parameters were not statistically significant, but episodes of hypocalcemia were not severe and could be managed without clinical emergencies [48]. In another study, 24 HD patients affected by severe secondary hyperparathyroidism (PTH > 800 pg/ml) and low BMD (DXA T score < -2.5) received a single 60 mg subcutaneous (s.c.) dose of denosumab to evaluate the short-term effects of its co-administration with calcitriol on PTH and parathyroid glands. PTH levels and parathyroid gland volume significantly decreased in the treated but not in the control group (calcitriol therapy only) in which a progressive increase occurred [49]. Femoral neck and lumbar spine BMD also significantly increased. The most common adverse event in the treatment group was hypocalcemia, which occurred in spite of the concomitant increasing doses of calcitriol, but for the most part it was sensitive

to simple oral calcium supplements. Serum levels of phosphate also decreased. Notably in this study, the dose of calcitriol reached an average of 16 mcg/week for a few months and could thus be regarded as the drug responsible for the biochemical and instrumental changes described. Accordingly, we should consider that in this study a major advantage with denosumab was the reduction of serum levels of both calcium and phosphate which allowed to up-titrate the doses of vitamin D.

Table 2 schematically reports the indications, contraindications, pharmacokinetic data, collateral effects, dosing and standard duration of therapy with denosumab. As a whole, denosumab represents a potentially useful drug for bone disease in renal patients. However, we lack specific trials indicating the specific renal clinical conditions in which its advantages are superior to its potentially negative side effects. Moreover, given the antiresorptive action, its administration seems best indicated for patients with increased bone turnover or resorption, i.e. severe secondary hyperparathyroidism. Conceivably, while also patients with mixed lesions could gain from denosumab therapy, those with low turnover should not receive it. A specific point to appreciate and verify is that, given the long half-life of the drug, hypocalcemia (even asymptomatic) may be prolonged and cause parathyroid gland hypertrophy with the risk of rebound hyperparathyroidism. Further, a warning in general and in renal patients in particular could be necessary regarding the non-bone related effects (on the immune system and inflammation). In particular, given the involvement of the RANK/RANKL/OPG system with the process of calcification and with atherosclerosis [50], also these systemic effects should be considered.

Romosozumab

Romosozumab is a human monoclonal immunoglobulin (Ig)G2 antibody produced in a mouse cell line by recombinant DNA technology. It targets sclerostin thus exerting both anabolic and anti-resorptive actions (Fig. 4). Sclerostin is a 23 kDa glycoprotein produced by osteocytes that, by binding low density lipoprotein receptor related protein 5/6 (LRP5/6) and its co-receptor frizzled, antagonizes the canonical, beta-catenin dependent, Wnt signaling in osteoblasts and inhibits bone formation [17]. The Wnt signaling pathway in osteoblasts is determinant to stimulate osteoblast differentiation and proliferation. Sclerostin, a Wnt antagonist, prevents bone formation by disabling the association of Wnt ligands with their co-receptors. Sclerostin also increases RANK-L expression in osteoblasts and thereby stimulates osteoclastic bone resorption [51]. Indeed, sclerostin gene (SOST) knock-out mice and SOST inactivating mutations in humans [52, 53] are both characterized by increased bone formation and sclerosis of the skeleton [54].

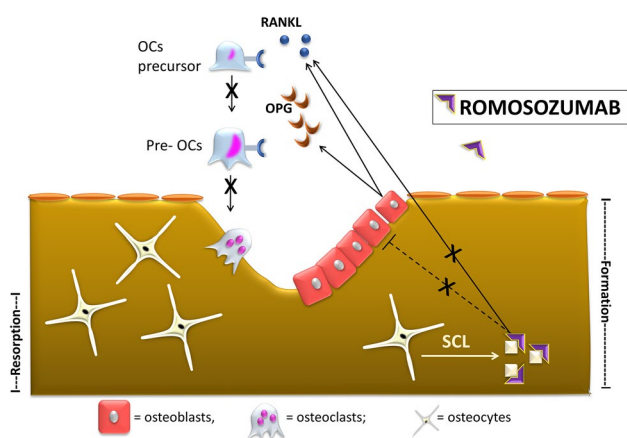


Fig. 4 Effects of romosozumab on bone. Romosozumab targets and binds sclerostin (Scl). Physiologically, Scl reduces osteoblastogenesis and promotes osteoclastogenesis. Thus, Scl inhibition leads to both anabolic (increased osteoblast activation) and anti-resorptive (reduced osteoclastogenesis) effects

It can be noted that available drugs for OP treatment either inhibit bone resorption (bisphosphonates, denosumab) or increase bone formation (teriparatide), while the most powerful drug for OP would be one that both reduces bone resorption and stimulates bone formation, an effect theoretically obtainable by inhibiting sclerostin. Indeed, experimental studies with anti-sclerostin antibodies, in different animal models, invariably demonstrated an increase in bone mass and an improvement in bone strength at all skeletal sites examined [55]. Histologically there was evidence of increased bone formation in trabecular, endocortical, and periosteal surfaces with eventual increment of trabecular and cortical thickness. Further, the mineral apposition rate and mineralizing surfaces were also increased suggesting that sclerostin antibodies actually increased both activity and number of osteoblasts [54, 56]. Since a decrease of osteoclast surface was also observed, its anabolic effect did not lead to increased bone resorption. Pre-clinical observations also evidenced that the gain in bone mass was due to stimulation of bone formation in the earlier period combined with reduced osteoclastic activity later on [57]. Romosozumab is the first monoclonal humanized sclerostin neutralizing antibody, developed to treat OP, for which we now have a phase III clinical study. The first placebo-controlled phase I trial, in human volunteers, enrolled 6 healthy men and 42 postmenopausal women [58]. They received single increasing doses of romosozumab subcutaneously (from 0.1 to 10 mg/kg) or intravenously (1 or 5 mg/kg) or placebo and were then followed for 85 days for an analysis of safety, pharmacokinetics and pharmacodynamics. With the highest dose, bone formation markers (serum procollagen type 1 N-propeptide or PINP, bone AP, and osteocalcin) increased rapidly and progressively (+176%) and

returned to baseline values after roughly 2 months. Bone resorption marker (serum C-telopeptide, CTX) decreased by a maximum 54% of baseline values 2 weeks after injection and returned to baseline after 2 months. Increment in PINP, bone AP, and osteocalcin and decrease in CTX were dose dependent. Similarly dose dependent was the increase in BMD, with the largest amount occurring in lumbar spine (5.3%) and total hip (2.8%) 85 days after a s.c. dose of 10 mg/kg. Non-linear pharmacokinetic profiles were recorded, with peak serum concentrations in the first week following s.c. administration. Further, in the highest s.c. and i.v. dose groups, serum concentrations of romosozumab declined biphasically. A single treatment-related serious adverse event of non-specific hepatitis was reported. No death occurred. Mean serum ionized calcium transiently decreased by only 4%. The reduction was not associated with symptoms and calcium returned to baseline during the follow-up period.

A phase II, placebo-controlled study with romosozumab was conducted in 419 postmenopausal women, aged 55–85 years, with low BMD (T score -2.0 or less at the lumbar spine, total hip, or femoral neck and -3.5 or more at each of the three sites) [59]. Participants were randomly assigned to receive romosozumab 70, 140, or 210 mg every month, or 140 or 210 mg every 3 months for 12 months. An open-label active comparator (alendronate or teriparatide) and a placebo group were also included. The primary end point was the percentage change from baseline in BMD at the lumbar spine at 12 months. Secondary end points included percent changes of BMD at other sites and of markers of bone turnover. At month 12, participants in the romosozumab group, compared to the placebo, had a significant increase in bone mineral density at the lumbar spine regardless of the dose or dose frequency. In addition, the romosozumab groups, compared to the placebo, had a significant increase in BMD at total hip and femoral neck. The largest BMD gain was observed with the 210 mg monthly dose which was significantly greater than that in the alendronate/teriparatide groups. Romosozumab was associated with a large increase in BMD at total hip and femoral neck. In all romosozumab groups, bone-formation markers (PINP, osteocalcin and bone AP) increased rapidly (at week 1), with peak increment at month 1, and returned to baseline or fell below baseline values between months 2 and 9. The level of the bone-resorption marker CTX decreased rapidly (in the first week) and remained below baseline values at month 12. Finally, in the phase III, double-blind, prospective, placebo controlled study, 7180 postmenopausal women who had a T score of -2.5 to -3.5 at the total hip or femoral neck were enrolled [60]. The primary endpoint was the cumulative incidence of new vertebral fractures at 12 and 24 months. Secondary end points included non-vertebral and clinical fractures (a composite

of non-vertebral and symptomatic vertebral). Patients were randomly assigned to receive subcutaneous injections of romosozumab 210 mg or placebo, monthly for 12 months; thereafter, both the placebo and the treatment group were shifted to an anti-resorptive drug, denosumab, administered as maintenance therapy, at the dose of 60 mg s.c., every 6 months. At 12 months, the risk of new vertebral fracture was 73% lower in the romosozumab group. BMD increased at 6 months, and at 12 months the percent change was greater with romosozumab than with placebo at the lumbar spine, femoral neck and total hip. The bone-formation marker PINP increased rapidly in the romosozumab group (maximum peak on day 14) and returned to baseline levels by 9 months. The bone-resorption marker β -CTX decreased early during treatment (maximum decline on day 14) and, at 12 months, values were lower than in the placebo group. Serious adverse events that were potentially indicative of hypersensitivity (dermatitis, allergic dermatitis, and macular rash) occurred in 7 patients in the romosozumab group. Injection-site reactions, mostly of mild severity, were reported in 187 patients (5.2%) in the romosozumab group and in 104 (2.9%) in the placebo group. Two events consistent with the definition of osteonecrosis of the jaw occurred in the romosozumab group only. The main pharmacological and clinical data pertinent to romosozumab are summarized in Table 2.

As a whole, this monoclonal antibody against sclerostin increases bone formation, decreases bone resorption and lowers the risk of vertebral and clinical fractures in postmenopausal OP women. Theoretically, it could be employed also in renal patients, but no pharmacokinetics data are available in the presence of reduced GFR. Its specific anti-sclerostin action points to a useful employment in particular in cases with adynamic bone disease.

Another aspect to consider when dealing with this drug is the possibility of extra-skeletal effects, in particular on vascular calcification. Clinical observations in hemodialysis patients showed higher serum sclerostin levels in those with aortic valve calcifications and upregulated sclerostin mRNA expression in calcified aortic valves explanted for clinical indications [61]. Similarly, in end-stage renal patients at time of transplantation, serum sclerostin correlated positively with vascular calcifications, but tissue expression of sclerostin was not evident in calcified epigastric arteries [62]. We can speculate that higher circulating levels of sclerostin are associated with osteoblast inhibition and low bone turnover, and, hence, with vascular calcifications. Alternatively, increased tissue levels could inhibit calcification by inhibiting activity and trans-differentiation of the osteoblast-like cells (i.e. calcifying vascular smooth muscle cells) responsible for calcification. Therefore, the final effect of sclerostin inhibition with romosozumab on vascular calcification is not predictable. Its anabolic effect

on adynamic bone could improve calcium and phosphate balance and then vascular calcification, while local reduction could favor it. In any case, romosozumab by interacting systemically and/or locally with the RANK/RANKL/OPG system and the Wnt pathway could definitely affect vascular calcifications and mortality [61, 63]. Accordingly, the link between vascular calcification and romosozumab therapy must be regarded as a new, still unexplored, field of clinical research.

Future perspectives

Teriparatide, denosumab and romosozumab are very new drugs for the nephrologist. They modify the activity of the individual bone cells and can drive their normally coupled activity toward different balances. Nephrologists should quickly learn if and how to employ them in specific ROD types. Specifically, since the most frequent bone lesion described in CKD-5D patients is low turnover bone and since we can generally cure high turnover bone through medical or surgical therapy, a “wish come true” trial could focus attention on adynamic bone disease. One could propose an ideal prospective randomized trial exploring hard outcomes (fractures, vascular calcifications and cardiovascular mortality) in incident dialysis patients with bone biopsy-proven adynamic bone and randomized to standard therapies (placebo) or teriparatide for 1 year, followed by denosumab for a second year. Alternatively, also romosozumab could be employed, but we lack any available clinical evidence for its use in renal patients.

Conclusions

Bone disease in renal patients has now clearly become transformed into a complex bone endocrinopathy at the heart of CKD-MBD, with a significant potential link to cardiovascular disease. The new biologicals established for OP have direct effects on the activity of bone cells with effects that could be tailored for the treatment of some specific ROD types. However, the evaluation of systemic effects is also warranted, given the complexity of bone disease in renal failure.

Compliance with ethical standards

Conflict of interest LT: no conflict of interests to declare; MP: no conflict of interests to declare; SR: no conflict of interests to declare; MML: no conflict of interests to declare; AC: consultant for FMC; SM: clinical investigation fundings from Amgen.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent For this type of study formal consent is not required.

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