Monoclonal antibodies for treating osteoporosis

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ABSTRACT

Introduction: Osteoporosis is the most widespread skeletal disease requiring innovative therapeutic strategies for its management. The understanding of RANKL and sclerostin role in bone cell biology is completely changing the therapeutic scenario. RANKL supports osteoclast formation and activity and it is mainly produced by cells of the osteoblastic lineage. Sclerostin, an antagonist of the Wnt pathway, has a key role in bone formation and it is mainly secreted by osteocytes. High levels of RANKL and sclerostin have been detected in osteoporosis, leading to the production of antibodies able to bind and neutralize their activity: Denosumab and Romosozumab, respectively.

Areas covered: In this review, the authors overview and discuss literature data on Denosumab and Romosozumab for the treatment of osteoporosis. Clinical studies indicate that long-term treatment with Denosumab causes a continuous increase of the bone mineral density (BMD) with low incidence of adverse effects. Romosozumab treatment determines an increase of bone formation and an improvement of BMD; however other studied are needed to better evaluate the adverse effects.

Expert opinion: Denosumab and Romosozumab show beneficial effects on osteoporosis treatment. The different mechanisms of action compared to the existing anti-osteoporotic drugs may permit alternative strategies for osteoporosis treatment.

Keywords: osteoporosis, RANKL, Denosumab, Sclerostin, Romosozumab

HIGHLIGHTS

1. The discovery of the role of RANKL and sclerostin led to development of two important monoclonal antibody for the therapy of osteoporosis: Denosumab and Romosozmab.

2. Denosumab is the only monoclonal antibody approved by FDA for the therapy of osteoporosis. Clinical studies indicate that the treatment of osteoporotic patients with Denosumab for 10 years causes a continuous increase of the bone mineral density (BMD) with low incidence of adverse effects.

3. Romosozumab treatment determines an increase of bone formation and an improvement of BMD. However recent studies highlighted a risk for the development of cardiovascular adverse events, thus further investigation are needed to obtain the approval in clinical practice.

4. The therapeutic efficacy of Denosumab is quickly inverted after treatment discontinuation, thus leading to fast loss of its curative effects. Consequently, spontaneous single or multiple vertebral fractures have been observed during the discontinuation period.

5. Denosumab and Romosozumab have a major patients' compliance compared to the classical therapeutic approaches for osteoporosis as both the antibodies require comfortable administrations.

1. INTRODUCTION

Osteoporosis is characterized by skeletal fragility and increased fracture risk as consequence of an altered bone remodelling [1]. In physiological conditions bone undergoes a continuous process of "renewal" thanks to the coupled bone resorption activity by osteoclasts (OCs) and bone formation counteraction by osteoblasts (OBs) [2-4]. This process is known as bone remodelling and it is arranged within temporary anatomical structures, identified as basic multicellular units (BMUs). Furthermore, bone can also be shaped by bone modelling, a process characterized by the uncoupling of bone resorption or formation [2-4]. Bone modelling is necessary for shaping bone architecture following mechanical load/strains, and it is linked to individual hormonal and genetic factors. It has been lately reported that bone modelling occurred both before puberty as well as in adult life [5]. Active modelling occurs in different sites, such as distal radius, tibia, ribs, and femoral diaphysis in the elderly [6,7]. In bone disease, as osteoporosis, the quantity of bone resorbed by OCs exceeds the amounts deposed by OBs with consequent damages of skeletal architecture and decreased bone strength [8]. The biological studies led to the understanding of the mechanisms underlying bone remodelling and thus to the identification of new pharmacological targets suitable to improve bone health in osteoporosis. In detail, the discovery of the role of Receptor activator of nuclear factor kappa-B ligand (RANKL) [9] in supporting the osteoclastogenesis, and of sclerostin [10] in inhibiting the osteoblastogenesis, led to the design of two monoclonal antibodies anti-RANKL (Denosumab) and anti-sclerostin (Romosozumab) for the therapy of osteoporosis. In this review, we overview the role of RANKL and sclerostin in physiological and pathological bone remodelling as well as the current use of Denosumab and Romosozumab.

2.RANKL

The OC activation and differentiation is under the control of a family of biologically related tumor necrosis factor (TNF) receptor (TNFR)/TNF like proteins: OPG, RANK and RANKL [9,11]. RANKL is expressed by bone marrow stromal cells, OBs, osteocytes and activated T cells [9,12].

RANKL together with macrophage colony-stimulating factor (M-CSF) promotes the fusion of monocyte-macrophage precursors to form mature and active OCs [11]. RANKL also inhibits OC apoptosis [9]. Consistently, RANKL knockout mice shows severe osteopetrosis and complete absence of OCs [13]. RANKL expression can be up-regulated by glucocorticoids, interleukin (IL)-1, IL-6, IL-11, IL-17, and Tumor necrosis factor-α (TNF-α) [12,14]. Moreover, RANKL pro-osteoclastogenic activity can be increased by other pro-osteoclastogenic cytokines, as TNF-α and LIGHT/TNFSF14 (homologous to Lymphotoxins exhibiting Inducible expression and competing with herpes simplex virus Glycoprotein D for herpes virus entry mediator [HVEM], a receptor expressed by T lymphocytes) [15,16]. The receptor for RANKL is RANK, a member of TNF-R superfamily; it is a transmembrane heterotrimer expressed on the surface of pre-osteoclasts and mature OCs [9]. The activation of RANK by its ligands leads to the expression of genes required for OC differentiation, activation of bone resorption and OC survival [17]. Osteoprotegerin (OPG) is a soluble glycoprotein secreted by OBs, osteocytes, T- and B-cells that acts as a "decoy receptor," blocking the binding of RANKL to its receptor RANK and leading to inhibition of OC formation and activity [18]. The ratio between RANKL and OPG is thus an important issue for skeletal homeostasis. In fact, if the ratio moves in favor of RANKL, bone remodeling shifts towards an increase of osteoclastogenesis with consequent bone diseases, such as post-menopausal osteoporosis, genetic disorders, inflammatory disorders, and cancer-related bone loss [19].

3. DENOSUMAB

Denosumab is a fully human monoclonal antibody to RANKL, the first antibody approved by the United States FDA for the treatment of osteoporosis or in patients at high fracture risk [20-23]. It binds with high affinity and specificity to RANKL, simulating OPG inhibitory effect with consequent fast suppression of bone resorption. The approval by FDA arose next a large 3 year clinical trial – the Fracture Reduction Evaluation of Denosumab (FREEDOM) study [24]. In the multicentre, randomised, double-blind, placebo-controlled, phase 3 FREEDOM trial,

postmenopausal osteoporotic women aged 60-90 years were enrolled in 214 centres in North America, Europe, Latin America, and Australasia and were randomly assigned (1:1) to receive 60 mg subcutaneous Denosumab or placebo every 6 months for 3 years. In this study, it has been reported that Denosumab determined an increase of BMD at five diverse bone sites – hip 6.0%, lumbar spine 9.2%, throchanter 7.9%, femoral neck 4.8%, and 3.5% at 1/3 distal radius [24]. In parallel, new vertebral, hip, and non-vertebral fractures were decreased by 68%, 40%, and 20%, respectively [24]. The non-vertebral fractures did not include fractures of the skull, mandible, face, fingers, toes, or metacarpals, as they are not linked to reduced BMD; pathologic fractures and those that were associated with severe trauma.

Furthermore, enhancement of volumetric BMD in the cortical and trabecular compartments of the tibia was also measured. Cortical porosity and thickness, which represent indicators of bone quality, were also reported to increase with Denosumab compared to the alendronate treatment [25]. Very recently, it has also been reported that Dual-energy X-ray absorptiometry (DXA) scans revealed progressive increases of Trabecular Bone Score (TBS) from baseline at 12, 24, and 36 months following Denosumab treatment [26]. A remarkable decrease in CTX (crosslinked telopeptide of type 1 collagen), was observed during 3 years follow-up of Denosumab treatment, reflecting its anti-resorptive action [24]. In addition, a sustained decrease of type 1 collagen amino-terminal pro-peptide (P1NP) and bone-specific alkaline phosphatase (BSAP) was reported to occur after the first injection of Denosumab both in humans and in monkeys [24,27-29]. Histomorphometry evaluations demonstrated a strong, continued inhibition of bone turnover, with normal bone microarchitecture and with no damages on mineralization [30].

Denosumab was not reported to have negative effects on fracture healing in the FREEDOM trial, despite the timing of treatment [24]. Consistently, no negative effects on healing have also been shown in pediatric subjects [31, 32].

All patients who have done the FREEDOM trial without suspending the treatment or missing more than one dose of Denosumab were qualified to take part in the open-label, 7-year extension, in

which all patients received the antibody [33]. The study also includes women who received 3 years of placebo and transitioned to Denosumab in the extension (crossover group). The primary outcome was safety supervising, whereas the secondary outcomes were finalized to monitor new fractures as well as BMD. Few cases of osteonecrosis of the jaw, atypical femoral fracture, and hypocalcemia were reported. Interestingly, in the long-term group, BMD augmented from FREEDOM baseline by 21.7% at the lumbar spine, 9.2% at total hip, 9.0% at femoral neck, and 2.7% at the one-third radius. In the crossover group, BMD augmented from extension baseline by 16.5% at the lumbar spine, 7.4% at total hip, 7.1% at femoral neck, and 2.3% at one-third radius. Denosumab administration for up to 10 years was linked to low incidence of unfavourable events, low fracture rates respect to that experienced throughout the original trial, and sustained increase in BMD without plateau [33].

CTX and P1NP median serum levels were decreased during the 7 years of the extension in the long-term group [33]. In the crossover group, CTX and P1NP median serum levels diminished quickly after the initial administration of Denosumab, as observed in subjects receiving the antibody during FREEDOM [33]. Decreases in the crossover group were maintained during 7 years of treatment and were similar to the results observed for the long-term group during the first 7 years of Denosumab administration. Also BSAP showed the same trend.

3.1 Combining Teriparatide with Denosumab

The skeletal responses following the simultaneous use of Denosumab with teriparatide have been also evaluated in the Denosumab and Teriparatide Administration (DATA) trial. In the first year of combined therapy, the BMD was higher at 6 and 12 months in both the hip and the spine than measured with either drug alone [34]. This BMD gain was preserved but did not progress more during the second year of combined therapy [35]. During the 24 months of therapy in the combination group, the levels of CTX and P1NP in the sera were similar to those of the Denosumab only group, suggesting that Denosumab completely blocked both the resorption and increase in

formation deriving from teriparatide monotherapy. Serum levels of osteocalcin, an additional marker of bone formation that augments in response to teriparatide, also declined following the combination therapy, even if more slowly than with Denosumab alone.

The combined therapy of Denosumab and Teriparatide was also tested in Italy [36]. It consisted of commencement with teriparatide 3 months following the first injection of Denosumab. During the first 3 months of unopposed Denosumab therapy, serum CTX and P1NP declined as supposed. Following the subsequently 9 months on combined therapy, CTX switched to baseline serum levels, whereas P1NP levels increased over baseline, even if did not reach the higher levels of teriparatide monotherapy.

High-resolution peripheral quantitative computed tomography (HRpQCT) at 12 and 24 months in the DATA Trial demonstrated that total volumetric bone mineral density (vBMD) at the radius and tibia, trabecular vBMD at the radius, and cortical vBMD at the tibia augmented more in the combined therapy than both single groups [37, 38]. In the combined therapy also cortical thickness at the tibia augmented more than each monotherapy. Cortical porosity was enhanced with teriparatide mainly in the radius, but not in the Denosumab or the combination therapy. At the tibia Trabecular vBMD was similarly improved in all groups, while trabecular vBMD at the radius augmented more in the combined therapy than the each monotherapies. At both the radius and tibia finite element analysis-estimated strength increased or was preserved both by combined therapy and monotherapies [38].

However, the DATA Trial and the Italian study were too little to establish if the mixture of teriparatide and Denosumab determined a better or earlier decrease in fracture risk.

3.2 Bone Turnover Rebound and Post-discontinuation Effects

Differently from bisphosphonates (BPs) which integrate into hydroxyapatite and show prolonged half-lives, the therapeutic efficacy of Denosumab is quickly inverted after the treatment discontinuation [39]. Thus, it is possible that upon Denosumab withdrawal, RANKL is immediately

available and thus OCs rapidly differentiate and reabsorb bone matrix. Furthermore, it is important to consider the key role of osteocytes in RANKL secretion. In detail, it has been reported that osteocytes neighbouring areas of bone damage secrete RANKL, which is responsible of OC recruitment from up to 300 mm away [40]. Therefore, it is probable that following Denosumab withdrawal these particular areas could promptly reactivate. In parallel it is important to remember that after Denosumab withdrawn the remodeling activity of numerous BMUs re-start simultaneously [41].

In post-menopausal women, Denosumab discontinuation determines a rebound of CTX and P1NP to levels 60 and 40% over the pre-treatment amounts, respectively, and remains sustained for about 2 years [39]. In children bone turnover rebound seems to be stronger, of minor duration, and more frequently coupled to hypercalcemia. In fact, it has been reported that in a child treated for fibrous dysplasia, CTX serum levels rebounded to 250% over the baseline, thus requiring BP administration to control hypercalcemia; CTX then came back to baseline after 5 months [31]. Otherwise, hypercalcemia arising from discontinuation of Denosumab is reported for an adult subject treated with long-term Denosumab therapy [42], and five pediatric patients [31, 43-46]. In children this adverse effect can be associated to the elevated baseline bone turnover, which possibly determines a major rebound post-Denosumab discontinuation. This effect is important because treatment with Denosumab or with BPs in children, determines the development of dense metaphyseal bands on radiographs, although histological analysis in a child with fibrous dysplasia showed a persistence of epiphysis activity during and after Denosumab administration [47]. Importantly, the advantage of Denosumab is that after its interruption the sclerotic metaphyseal bands rapidly disappear, thus maybe contributing to development of post-discontinuation hypercalcemia. Bone turnover reversibility after Denosumab discontinuation also leads to fast loss of its curative effects. Patients discontinuing Denosumab during the FREEDOM trial vanished the increased bone density reached during treatment over a 1-year period [48]. This phenomenon can explain the observation in the post-marketing period: spontaneous single or multiple vertebral

fractures developed throughout the discontinuation period in post-menopausal patients [49-52]. On the other hand, reversibility represents an intriguing characteristic in children care disorders because brings the prospective for full rescue. All these findings imply that Denosumab effect on bone cells could be cytostatic but not cytotoxic.

3.3 How explain the continuous increase of BMD using Denosumab?

Denosumab treatment determines a remarkable and continued boost in BMD by mechanisms which are at present under investigation. Firstly, the reduction of markers of bone turnover suggests that bone remodelling is almost totally blocked throughout Denosumab treatment. This finding implies that bone modelling could be involved in the increased BMD associated to Denosumab treatment as suggested by studies on osteoporotic animal models [29,53]. In detail, CTX and soluble bone alkaline phospatase levels strongly decreased following Denosumab treatment of ovariectomized cynomolgus monkeys for 16 months [54]. This timing corresponds to 4 years of bone remodeling in an adult human. Despite the reduced bone turnover, Denosumab treatment increases Bone Mineral Content (BMC) and vBMD in both trabecular and cortical bone, and in total vBMD compared with sham-operated monkeys. Continuous modeling-based bone formation despite suppression of bone remodeling has been reported by Ominsky et al. [29]. Thus, Denosumab increased cortical thickness of long bones due to modeling-based slight formation of new bone. Secondly, in osteoporosis the impaired bone-remodelling blocks BMUs from the attainment the highest levels of mineralization (secondary mineralization) [55]. The majority of anti-resorptive drugs supports mineralization by prolonging the time-lapse of secondary mineralization [29,57]. In the case of Denosumab, the effect could be stronger and/or more persistent than that associated with BPs, leading to few new bone modeling units and concurrent filling in of preexisting resorption cavities [58,59]. Thirdly, latest report has revealed a possible anabolic role of Denosumab associated to RANKL-dependent reverse signalling in OBs [60]. In detail, recent *in vivo* and *in vitro* studies have shown that in OBs stimulation of transmembrane RANKL by W9 peptide can activate an anabolic response [60]. W9

binding to osteoblast-anchored RANKL activates the p38 MAPK pathway and SMAD1/5/9 phosphorylation, leading to the increase of numerous OB growth factors or their receptors, such as bone morphogenic protein 4 (BMP4), insulin-like growth factors 1 and 2 (IGF-I and IGF-II), and fibroblast growth factor receptor 2 (FGF-2). However, further investigations are required to deepen the role of this reverse signalling in bone mass accrual following Denosumab treatment. Fourthly, it has been recently reported that bone marrow adipocytes can modulate osteoclastogenesis through the production of RANKL thus possibly modulating osteoclastogenesis in the BMU [61]. Therefore, it is possible that blocking RANKL in adipocytes also might partially contribute to the pro-osteogenic effect of Denosumab. However, *in vivo* studies are needed to better explore this issue.

3.4 Current studies reporting Denosumab treatment in other bone disease.

High levels of RANKL have been found in numerous bone diseases [62-65], thus sustaining the use of Denosumab in different pathologies. At moment Denosumab is approved for bone loss associated with sex steroid deprivation therapy, as a results of 2 trials demonstrating BMD increase following Denosumab treatment of men receiving androgen deprivation therapy for prostate cancer [66], and of women receiving adjuvant aromatase and inhibitors for breast cancer [67]. Moreover, Denosumab is also approved for the prevention of skeletal related events associated to breast and prostate tumors [22,23,68] as well as for hypercalcaemia [69].

Denosumab is the only medication currently indicated for treatment of giant cell tumors in adults and skeletally mature adolescents [70]. The antibody was also tested in Osteogenesis Imperfecta, the mainly common form of primary osteoporosis. Improvement of BMD was observed in type VI osteogenesis imperfecta [71,72], whereas other types of this pathology require further study to establish the optimal dose and frequency able to inhibit OC activity [45,73]. There are also studies ongoing for Juvenile Paget's disease, a disorder arising from mutation inactivating *OPG* gene [74], evaluating Denosumab efficacy [46].

Denosumab has been tested as a possible therapy for some benign fibro-osseous lesions affecting pediatric patients, such as fibrous dysplasia [31, 47], central giant cell granuloma [75], and spinal aneurysmal bone cysts [76, 77].

4. SCLEROSTIN

The two human bone phenotypes of sclerosteosis [78] and van Buchem's disease [79], both characterised by increased bone mass and intrinsic resistance to fractures resulted from the functional loss of the Wnt/βcatenin signaling inhibitor sclerostin. Wnt/βcatenin signaling pathway orchestrates the differentiation of mesenchymal stem cells inhibiting chondrogenic and adipogenic differentiation and inducing osteoblastogenesis [revised in 80]. Wnt/βcatenin signaling also stimulates survival of OBs and osteocytes, and reduces osteoclastogenesis through the increase of the expression in OBs and osteocytes of OPG [10]. Thus, activation of this pathway is crucial for increased bone formation and decreased resorption. Osteocytes are critical players in the modulation of the canonical Wnt/βcatenin signaling as responsible of the secretion of sclerostin, a protein encoded by the *Sost* gene largely expressed by mature osteocytes but not by early osteocytes or OBs [81]. Moreover, genetic deficiency of *Sost* in mice as well as neutralizing antibodies for sclerostin mimicked the high bone mass phenotype associated to human pathologies lacking sclerostin [78, 79, 82]. In contrast, over-expression of Sost/sclerostin decreases bone mass [83-86], as observed in several bone diseases thus supporting the use of anti-sclerostin antibody in their management [87-94].

5. ROMOSOZUMAB

The results obtained on animal models following sclerostin neutralization led to realization of two monoclonal antibodies neutralizing sclerostin in human subjects: Blosozumab and Romosozumab [95]. Blosozumab is an IgG4 humanized monoclonal anti-sclerostin antibody that gave good results

in Phase I and II trials. Otherwise, the Phase 3 fracture trial with blosozumab has been delayed indefinitely [95].

Romosozumab is an IgG2 monoclonal antibody created by humanizing a mouse sclerostin monoclonal antibody. Romosozumab neutralizes the activity of human, monkey, and rat sclerostin and has a high binding affinity for human sclerostin. Following the successful results of Phase I and II trials [96-98], several Phase III trials of Romosozumab are currently ongoing or have been recently concluded with encouraging results [99-102]. In detail, a Phase 3 placebo-controlled FRActure study in postmenopausal woMen with ostEoporosis (FRAME, ClinicalTrials.gov identifier: NCT01575834), is a multicenter, randomized, double-blind, placebo-controlled, parallel group study that compares the 1-year administration of Romosozumab followed by Denosumab with 1-year administration of placebo followed by Denosumab [99]. It has been enrolled 7.180 post-menopausal women (55–90 years-old), having a total hip or femoral neck BMD *T*-score of −2.5 to −3.5. The principal endpoints were the decrease vertebral fracture at 12 and 24 months. At 12 months of Romosozumab administration, vertebral fractures were decreased of about 73% (the incidence of vertebral fracture in the Romosozumab group was 0.5% as compared to 1.8% in the placebo group). At the end of 24 months, vertebral fracture risk was decreased by 75% in patients who received Romosozumab in the first year and Denosumab in the second year as compared to the group who received placebo in the first year and Denosumab in the second year. At 12 and 24 months no significant difference was found in non-vertebral fracture incidence between the two groups [99]. By 12 months there were significant increases in BMD in the total hip (6.9%), lumbar spine (13.3%), and femoral neck (5.9%). Following Romosozumab treatment, serum P1NP quickly augmented and came back to baseline by 9 months of treatment. Otherwise, CTX levels declined early and stayed low throughout the 12 months of treatment. Adverse events included mild injection site reactions in 5.2% of patients treated with Romosozumab, two cases of osteonecrosis of the jaw and one case of atypical femoral fracture. The first event of osteonecrosis appeared after 12 months of Romosozumab treatment in the context of ill-fitting dentures, whereas the second event appeared

after 12 months of Romosozumab treatment and one Denosumab dose after a tooth extraction and successive jaw osteomyelitis. Atypical femoral fracture occurred 3.5 months after the first dose of Romosozumab, but the patient had a history of prodromal pain at the site of fracture beginning before the enrollment. Thus, both these side effects developed in patients with confounding factors contributing to the problem or raising queries about causality.

The just completed study is the randomized, open-label, international multicenter STRUCTURE study (STudy evaluating effect of RomosozUmab Compared with Teriparatide in postmenopaUsal women with osteoporosis at high risk for fracture pReviously treated with bisphosphonatE therapy, NCT01796301) [100]. This study projected to investigate the consequence of a 12-month treatment with either Romosozumab or teriparatide on BMD after BP treatment. The study involved 436 postmenopausal women aged 55–90 years with osteoporosis (*T*-score ≤−2.5 at the lumbar spine, femoral neck, or total hip) who had taken an oral BP for at least 3 years before enrolment and, specifically, had taken weekly alendronate 1 year before screening. Patients also had to have a history of a vertebral fracture or a non-vertebral fracture after the age of 50 years. In the study, the mean patient *T*-scores were as follows: lumbar spine −2.2, total hip −2.9, and femoral neck −2.5. 436 patients were randomly assigned to Romosozumab (n=218) or teriparatide (n=218). 206 patients in the Romosozumab group and 209 in the teriparatide group were included in the primary efficacy analysis. The major endpoint of the STRUCTURE study was total hip BMD at month 12 [100].

The STRUCTURE study showed that Romosozumab significantly augmented total hip BMD (2.9%) and was better than teriparatide (−0.5%). Romosozumab also determined higher gains in lumbar spine BMD as compared to teriparatide (9.8% in patients on Romosozumab and 3.5% in patients on teriparatide). It is remarkable to note that, Romosozumab administration determined BMD increases in the cortical and integral compartments of the hip and improved the estimated hip strength (differently from teriparatide which determines a decline in the estimated hip strength). The frequency of adverse events was generally balanced between treatment groups. The most frequently

reported adverse events were nasopharyngitis (28 [13%] of 218 in the Romosozumab group *vs* 22 [10%] of 214 in the teriparatide group), hypercalcaemia (two [<1%] *vs* 22 [10%]), and arthralgia (22 [10%] *vs* 13 [6%]). Serious adverse events were reported in 17 (8%) patients on Romosozumab and in 23 (11%) on teriparatide; none were evaluated as treatment related. There were six (3%) patients in the Romosozumab group compared with 12 (6%) in the teriparatide group with adverse events leading to investigational product withdrawal [100].

A just published paper reports the results of the phase 3 ARCH (Active-contRolled fraCture study in postmenopausal women with osteoporosis at High risk) study (NCT01631214) [101]. This multicenter study enrolled 4093 postmenopausal women (mean age 74) with osteoporosis and high fracture risk. Subjects were randomized to receive Romosozumab or alendronate for 12 months (double blind period), followed by open label alendronate in both groups for additional 12 months. After 24 months of treatment, in postmenopausal osteoporotic women, Romosozumab followed by alendronate significantly increased BMD and decreased the risk of new vertebral (48%), clinical (27%), non-vertebral (19%), and hip fracture (38%) respect to alendronate alone. Romosozumab enhanced the levels of the bone-formation marker P1NP and reduced levels of the bone-resorption marker CTX within 12 months. After alendronate transition the levels of P1NP and CTX decreased. Adverse events were similar between groups, although the cardiovascular events seems to have a major incidence in Romosozumab group.

There is in progress a phase 2 clinical trial with Romosozumab for changes in BMD in men with osteoporosis (BRIDGE, NCT02186171) [102].

5.1 Possible complications of Romosozumab treatment

One of the uncertainties of sclerostin inhibition is the development of bone overgrowth and skeletal deformities as seen in sclerosteosis and van Buchem's disease, conditions with homozygous mutations. Encouragingly, heterozygous subjects with these mutations have modest levels of sclerostin, augmented bone formation, and bone mass but no bone overgrowth [103-105]. In the

FRAME study, the occurrence of hyperostosis was similar between the Romosozumab treatment group and the placebo group [99].

Another concern connected to pharmacological sclerostin inhibition is the probability of Romosozumab extraskeletal effects, because Wnt signaling pathway plays a key role in the development and homeostasis of numerous organs and tissues [106]. Certainly the secretion of sclerostin is limited to osteocytes within the musculoskeletal system; therefore, theoretically, the extraskeletal effects of sclerostin should be minimal.

However, recent studies demonstrated the pro-angiogenic role of sclerostin on Human Umbilical Vein Endothelial Cells (HUVEC) [107]. Additionally, literature data reported that patients with chronic kidney disease displaying vascular and aortic calcifications showed high serum sclerostin levels; moreover, sclerostin was found in vascular tissue undertaking calcification [108]. Notably, patients with sclerosteosis or van Buchem's disease did not show enlarged risk for cardiovascular disease [109]. In the FRAME trial, cardiovascular incidents were equilibrated between the Romosozumab treatment group and the placebo group [99]. Otherwise, data obtained from the ARCH study [101] have revealed that Romosozumab treatment is coupled with major cardiovascular events respect to alendronate, thus requiring further exploration.

Furthermore, since Romosozumab is an anabolic agents, other worries derived from its potential carcinogenic property. These fears are sustained by the findings that the bone-forming agent teriparatide led to the development of osteosarcomas in rats exposed to high doses of the molecule [110]. However, rat studies have not found an augment in the risk of carcinogenenicity associated to Romosozumab [111]. In the FRAME study, cancer risk was balanced and not diverse between the groups (Romosozumab 1.6% vs. 1.9% placebo) [99]. Therefore, at this moment experimental data lead to the exclusion of a carcinogenic potential for Romosozumab .

However, further studies are needed to resolve all the worries linked to Romosozumab use, to this extent the post-marketing period will be helpful.

6. EXPERT OPINION

Osteoporosis is a disease of the skeleton characterized by reduced bone mass and microarchitectural decline, with consequent decreased bone strength and augmented susceptibility to fracture. It is the most widespread skeletal disease, with a major occurrence in women. The disease concerns a broad part of the elderly population in industrialized nations and it is linked to an important socioeconomic burden as a consequence of increased fractures and mortality. Approximately 200 million subjects in the world experienced this disease, with about 14 million in the USA and 27.5 million in the EU in 2010. In accordance with the latter high prevalence, a cost of $37 billion in osteoporotic fractures was estimated for the EU in 2010. The classical therapies for osteoporosis include two different anti-resorpitive agents: BPs and selective estrogen receptor modulators (SERMs). BPs are analogs of inorganic pyrophosphate, are characterized by resistance to hydrolysis and high affinity for bone hydroxyapatite. These two properties determine their long skeletal half-life. Following adhesion to the bone surface, BPs are endocytosed by OCs, leading to the suppression of OC activity. However, the intrinsic mechanism of action also implies the long duration of their effect as BPs remain blocked in the bone matrix. Differently, SERMs are drugs designed to work as estrogen receptor agonists, thus substituting the absence of endogenous estrogen. SERMs inhibit OC activity, but also may have an anabolic activity on OBs. Recently, good results are deriving from the use of the only anabolic agent, teriparatide, which mimic the anabolic activity of PTH. Teriparatide limitations include the required daily injection of a drug that must be refrigerated, use restriction of no more than 2 years, and high costs. Recently, Abaloparatide, a synthetic analog of parathyroid hormone-related protein (PTHrP), has obtained the approval for the treatment of postmenopausal osteoporosis in women at high risk for fracture and presents some advantages compared to teriparatide. In detail, Abaloparatide does not need to be refrigerated and seems to determine a greater increases in BMD than teriparatide. Interestingly, hypercalcaemia incidence is lower in Abaloparatide respect to teriparatide group [112].

Furthermore, studies on Abaloparatide carcinogenesis in rats showed dose- and time-dependent osteosarcoma development with a comparable incidence to PTH(1–34) [113].

Recently, new pharmacological targets have been identified thanks to studies that have deepened the mechanisms underlying OC and OB activity: RANKL and sclerostin. The discovery of RANKL, a molecule promoting OC formation and activity, led to the production of Denosumab, an antibody able to neutralize RANKL, thus acting as an anti-resorptive drug. The FREEDOM extension trial gave important results both for the improvement of BMD as well as the low incidence of adverse effects. Furthermore, Denosumab has the compliance of patients as require few administrations/year. Moreover, it is important the continuous increase of BMD together with the quick reversibility of its effect. This last properties is important to preserve skeletal growth in the cure of children's diseases, but it also represents a problem for patients with a history of non-adherence, they may rapidly lose therapeutic effects if doses are missed or given late.

Sclerostin is an inhibitor of OB differentiation, thus the antibody neutralizing its effect, Romosozumab, has an anabolic effect. This molecule is younger compared to Denosumab so further studies are needed overall to be sure of its safety. As anabolic agent presents an advantage respect to teriparatide for the monthly treatment. Due to the uncertain consequences of continuous bone formation on the skeleton, there is apprehension for patients' exposure to long-term Romosozumab treatment. Thus, this drug could be used in cycle with anti-resorptive drugs, including Denosumab or BPs.

Although Denosumab and Romosozumab have a different mechanism of action, both improved bone health in osteoporosis. However, due the recent reports associating Romosozumab to cardiovascular adverse effects, the use of this molecule in the clinical practice remain only theory. Thus, Denosumab until now represents the only safe monoclonal antibody for osteoporosis management.

In the future a good chance to treat osteoporosis could derive from antibody improving muscle activity and consequently bone strength, such as bimagrumab [114]. This is an antibody blocking

the activin receptor, it is included in a phase 2 study [NCT02152761] in women and men older than 60 years who have osteoporosis after a hip fracture [114].

Therefore, although Denosumab and Romosozumab improve skeletal health further studies are needed to optimise their use. Moreover, the discovery of new mechanisms regulating bone cell activity could lead to the discovery of new antibody to treat osteoporosis.

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